# PEATLAND MICROBIAL COMMUNITY STRUCTURE AND FUNCTION ALONG A METAL CONTAMINATION GRADIENT IN SUDBURY, ONTARIO

by

Shanay Williams-Johnson

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science (MSc) in Biology

The Faculty of Graduate Studies
Laurentian University
Sudbury, Ontario, Canada

**THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE**
**Laurentian Université/Université Laurentienne**
Faculty of Graduate Studies/Faculté des études supérieures

| | |
|---|---|
| Title of Thesis<br>Titre de la thèse | PEATLAND MICROBIAL COMMUNITY STRUCTURE AND FUNCTION ALONG A METAL CONTAMINATION GRADIENT IN SUDBURY, ONTARIO |
| Name of Candidate<br>Nom du candidat | Williams-Johnson, Shanay |
| Degree<br>Diplôme | Master of Science |
| Department/Program<br>Département/Programme | Biology |
| Date of Defence<br>Date de la soutenance | October 26, 2016 |

**APPROVED/APPROUVÉ**

Thesis Examiners/Examinateurs de thèse:

Dr. Nadia Mykytczuk
(Supervisor/Directeur(trice) de thèse)

Dr. Nathan Basiliko
(Co-supervisor/Co-directeur(trice) de thèse)

Dr. Graeme Spiers
(Committee member/Membre du comité)

Approved for the Faculty of Graduate Studies
Approuvé pour la Faculté des études supérieures
Dr. Shelley Watson
Madame Shelley Watson

Dr. Derek MacKenzie Acting Dean, Faculty of Graduate Studies
(External Examiner/Examinateur externe)

Doyenne intérimaire, Faculté des études supérieures

(Internal Examiner/Examinateur interne)

**ACCESSIBILITY CLAUSE AND PERMISSION TO USE**

## Abstract

The Sudbury, Ontario region has had over a century of metal mining/smelter activity that has led to significant sulphur and metal deposition and this has negatively affected both freshwater and terrestrial ecosystems, including peatlands. Peatlands store organic materials, regulate nutrient turnover and act as a carbon sink for global climate change, yet relatively little is known in regards to the impact of the mining legacy of this region and the potential microbial communities affected. Eleven peatland sites (poor to intermediate fens) around Sudbury were chosen in order to study the microbial diversity and function that control decomposition and nutrient cycling. The analysis of microbial communities was accomplished via high-throughput sequencing of 16S rRNA genes in bacteria and archaea on the Illumina MiSeq platform, while the analysis of microbial function was conducted through the Sinsabaugh enzyme protocol and gas chromatography of in situ greenhouse gases. There was a difference across the site gradient with microbial diversity, community structure, and microbially mediated gas efflux differing between areas closest to current and historical smelters to areas 55-km away. There was also a difference within each peatland where vertical profiles in microbial enzyme function varied over four depths, with the surface depth having the highest enzyme activity. Metal impact and pH are major drivers of microbial diversity and community with pH driving metal availability. This is seen where the sites with the lowest pH having the lowest microbial diversity and unique communities, and sites with the highest pH having the highest microbial diversity and distinct communities. We can also deduce that microbial function differs over depth because of the difference between the aerobic and the anaerobic communities, where the aerobic communities appear to be more active. We can reason that methane efflux was higher in impacted sites because of the increased concentrations of Nickel, Copper, pH and possibly Sulphur creating restrains on microbially mediated gas effluxes through the inhibition of methane production.



***Keywords: Microbial community structure, bacteria, archaea, microbial diversity, taxonomic relatedness, Illumina MiSeq, peatlands, enzymes, $CO_2$ and $CH_4$ efflux, metals, industrial disturbance, Sudbury***

# Acknowledgements

I would like to thank my supervisors Dr. Nadia Mykytczuk and Dr. Nathan Basiliko for the incredible opportunity to work with them and for their continuous patience and support in all my pursuits. I would also like to thank my committee member Dr. Graeme Spiers for coming on board on short notice, and Dr. Daniel Campbell for his support, comments, and especially for the help he provided in data analysis and his insight on peatland ecology.

I am thankful for all the help and support I received from my fellow lab members and field assistants. I would like to specially thank Krystal Rancourt for her assistance in the field, and a special thank you to Emily Smenderovac, Caroline Emilson and Michael Carson for assisting me with R-Studio. I am also thankful for Amanda Lavallee, Nicole Valiquette, and Jesse Hoage for taking the time to help me with fieldwork.

I am grateful for all the help I received through the Living with Lakes Centre, especially from Karen Oman, Dr. John Gunn, and Dr. Tom Johnston. I am also thankful for the help and support I received from Kera Yucel, Vanessa Bourne, Galen Guo, Erik Emilson, and Alexandra Sumner, and the entire Living with Lakes family.

I am forever indebted to Jessica Arteaga for all her help and encouragement throughout my time at Laurentian University. I am thankful for her kindness and unwavering support, and for all her help in the field and in editing my writing.

I would like to thank two of my closest friends Evoné Walters and Marc Martin for always being my cheerleaders. I am also grateful for my family, especially my sister Jayani Williams for never letting me give up on myself. You have been there for me through the good and bad times, but most importantly, you believed in my dreams and in me even when no one else believed.

# Table of Contents

## List of Figures

## List of Tables

# 1 General Introduction

## 1.1 Peatlands and Their Key Functions

Northern peatlands comprise 2 classes of wetlands (bogs and fens) and contain at least 40 cm of organic matter (OM) that is formed when partially decomposing plant material accumulate over time (Moore and Bellamy, 1974; Gorham, 1991). This partially decomposed matter becomes preserved due to a combination of anoxia, low temperatures, nutrient limitations, complex substrates and low pH levels (Shotyk *et al.,* 1998; Suyama *et al.,* 2008). These factors result in constrained decomposition and mineralization rates (i.e. lower than rates of production) as soil microbial activities are inhibited (van Breemen, 1995; Shotyk et al. 1998; Freeman *et al.,* 2004). Peatlands act as natural environmental archives that allow for the analysis of changes in past ecosystems, climates and biological communities, especially in reconstructing past metal deposition in peat organic matter (OM) due to the ability of OM to strongly bind to metals (Shotyk, 1996; Shotyk *et al.,* 1998; Martinez-Cortizas *et al.,* 1999; Cole *et al.,* 1990; Suyama *et al.,* 2008). Peatlands accumulate nitrogen (N) and carbon (C), storing about one-third of the Earth's soil C, which is considerable since they only occupy about 3% of the Earth's land surface (Post *et al.,* 1982; Gorham, 1991; Limpens *et al.,* 2008). Because of their ability to sequester C, peatlands have become an important long-term sink for atmospheric carbon dioxide ($CO_2$), playing a very major role in global climate and future climate change-biosphere feedbacks. Despite the ability of peatlands to store large amounts of C in peat, they are also a major source of the greenhouse gas, methane ($CH_4$), which is produced when organic material decomposes in anoxic zones generally present in peatlands beneath the watertable (Matthews and Fung, 1987; Panikov, 1999; Smith *et al.,* 2004). Peatland ecosystems also function as a freshwater reservoir and indirectly yield approximately 10% of the world's freshwater supply (Joosten and Clarke, 2002). Many of these unique characteristics of peatlands are driven by a

large diversity of specialized plants, animals and microbes that are unique to these ecosystems (Andersen *et al.,* 2013b).

## 1.2 Classification and Characterizing Peatlands Types and Peat Horizons

Peatland ecosystems can be categorized into two distinct classes based on the dynamic interactions of their hydrology, vegetation, and nutrient status, and other peat characteristics: bogs and fens (Wells and Zoltai, 1985; Spitzer and Danks, 2006). Bogs are characterized by the prevalence of acidic peat, anoxic conditions, low nutrients and slow decomposition of organic material (Bragazza, 2012). *Sphagnum* mosses are important peat-forming plant species and are typically dominant in bogs because they thrive in acidic, low nutrient (e.g. N) conditions (van Breemen, 1995). Bogs are isolated from groundwater and receive their nutrients and water input from the atmosphere through rainfall and nutrient deposition (Bragazza, 2012). They are characterized by low pH (below 5.0) (Spitzer and Danks, 2006). In contrast, fens are characterized with higher peat pH, including occasionally even basic when connected to calcareous aquifers (Wells and Zoltai, 1985; Rydin and Jeglum, 2006). Unlike bogs, fens are not isolated from groundwater; instead, they receive their water and nutrients from groundwater as well as the atmosphere (Rydin and Jeglum, 2006), and thus although both bogs and fens play key roles in C storage, fens can play larger roles in affecting groundwater chemistry. Fen vegetation typically ranges from *Sphagnum* to sedge cover that is considerably correlated to the pore water chemistry across these ecosystems (Amon *et al.,* 2002).

Peatlands can be further classified chemically and also by nutrient levels. Peatlands can be classified using nutrient levels as eutrophic or very rich, mesotrophic or moderately rich, and oligotrophic or poor-fen (Mitsch and Gosselink, 2000). Chemically, peatlands can be classified into three main types, namely, minerotrophic or rich fen, mesotrophic or intermediate fen, and ombrotrophic or raised bogs (Mitsch and Gosselink, 2000). Fens are formed in areas where there is splitting in rock layers, which causes fractures to be formed in the landscape, resulting in a

hydrologic gradient that allows for the interaction of groundwater and soil (Amon, *et al.,* 2002). As previously mentioned, fens are groundwater fed, this groundwater can be derived from upward hydrologic gradients and so are a source of nutrients and cations (acid reducing) (Amon *et al.,* 2002). Poor fens and bogs are very similar from a chemical and vegetative perspective but from a hydrological standpoint, poor fens have a significantly different hydrologic regime that determines how the peat is formed (Szumigalski and Bayley, 1996). Peat accumulation along a successional stage in fens and bogs may suggest that true fens are the early successional stage, while bogs represent the later stage; and indeed many fens become bogs over time as peat becomes decomposed, and the low hydraulic conductivities restrict groundwater flow and as the relative surface of the peatland increases over time (Wells and Zoltai, 1985).

Peatlands are also often conceptually divided into two distinct layers based on Ingram's definition, namely the acrotelm and catotelm (Ingram, 1978; Ingram, 1983). These two layers can be distinguished by the degree/duration of waterlogging and the degree of peat decomposition (Ingram, 1978; Ingram, 1983). The acrotelm supports most of the biological activities that take place in a peatland, for example, the growth of vegetation such as mosses; this layer is oxic and is also affected by fluctuating water table throughout the year (Holden and Burt, 2003). The acrotelm layer is younger and more aerated and it is comprised of less decayed remains (Olid *et al.,* 2016). A deeper and thicker acrotelm occurs in sites with more extreme and frequent water-table drawdown, which may lead to further degradation in peatlands (Binet, 2013). The catotelm is distinguished as the layer that is permanently waterlogged and anoxic, and biota is primarily comprised of strict anaerobic microbes (Holden and Burt, 2003). The catotelm peat is more highly decomposed and dense (Olid *et al.,* 2016). In recent years, a third layer has been designated in the peatlands as the mesotelm (Clymo and Bryant, 2008). The mesotelm falls between the interface of the oxic and the anoxic layers, and is distinguished by fluctuating watertable and the simultaneous presence of $CH_4$ and $O_2$ (Clymo and Bryant, 2008; Andersen et al., 2013a).

## 1.3 Chemistry, Hydrology, and Vegetation of Peatlands

Fens span the range of poor to extremely rich, with pHs from ca. 5.0 to 8.0 respectively (Gignac *et al.,* 1991). The pH of these peatlands depends largely on groundwater source chemistry, hydrologic fluctuations and the interplay of vegetation and peat characteristics (Gignac *et al.,* 1991). Rich fens have far higher concentrations of anions such as bicarbonate, and cations such as calcium (Ca) (Zoltai and Vitt, 1995). The fluctuation of watertable levels affects the concentrations of the anions and cations present, for example, as driven by acidity and alkalinity generation by S oxidizers and reducers respectively (Gorham *et al.,* 1984; Zoltai and Vitt 1995). The higher alkalinity and calcium (Ca)-magnesium (Mg) concentrations, along with typically higher macronutrient (N and P) availability, play a major role in selecting for non-Sphagnum vegetation (Slack et al., 1980; Gorham *et al.,* 1984; Szumigalski and Bayley, 1996).

Peatland hydrology is influenced by several factors and the three potential sources of water inputs to a northern peatland are through precipitation, surface water and groundwater (Schiff *et al.,* 1998). The hydrology of a peatland is important in part because it influences how the site will respond to metals and acid inputs (Schiff *et al.,* 1998), which is the key focus of this thesis. For example, if fens have high Ca and Mg concentrations such as rich fens, there is stronger buffering against acid deposition and metal impact (Slack *et al.,* 1980), and in S rich sites, high and persistent water table positions lead to S reduction and alkalinity generation whereas low and/or fluctuating water tables lead to S oxidation and acidity generation typically coupled to increased metal solubility. Further, water table position is a key controller of microbial extracellular enzyme activities (addressed below in the introduction) and rates of peat accumulation and peat chemical characteristics.

The vegetation type present in a peatland strongly and dynamically influences the water chemistry and hydrology of that peatland (Mitsch and Gosselink 2000). *Sphagnum* mosses are keystone species in many peatlands globally (Rydin and Jeglum, 2006), dominating both bogs

and poor fens (Amon *et al.,* 2002). *Sphagnum* mosses are important peatland plants because of their ability to store and restrict the flow of large quantities of water and they also have the ability to acidify the pore water present in peatlands (Szumigalski and Bayley, 1996). These mosses are capable of driving peatland dynamics, shaping peatland habitats and stimulating peatland development by lowering pH and decomposition rates and by maintaining a high water table level (Chirino *et al.,* 2006). *Sphagnum* mosses require low nutrient concentrations and are not tolerant of many heavy metals (Chirino *et al.,* 2006). Because the tissues in these plants are decay resistant, a fibric layer of *Sphagnum* peat is created over time from slowly decomposing *Sphagnum* mosses; and both the living and decaying *Sphagnum* mosses hold substantial C in their biomass. *Sphagnum* mosses are often used as indicator species because they are the among the first species to show the effects of metal impact and other pollutants, unlike vascular plants that have a root system and a thick waxy cuticle to prevent metals from entering their cells (Tyler 1990). *Sphagnum* mosses do not have roots and they only have a small amount of cuticle, hence, they absorb their nutrients from the air or groundwater and this leaves them vulnerable to toxic elements (Tyler 1990). High concentrations of nickel (Ni) and copper (Cu) and other trace metals in the environment can lead to impact in plants, which negatively affects photosynthesis, autotrophic respiration, and overall production and can cause plants to eventually die (Michalak, 2006), leading to community shifts in polluted peatlands.

## 1.4 Peatland Microbiology

### 1.4.1 Microbial Communities and Diversity

The majority of the microbial taxa that are representative of northern peatlands have only been recently described in the literature (Dedysh, 2011). A selection of these has included members of the phyla Acidobacteria, Planctomycetes, Actinobacteria, Bacteroidetes, Verrucomicrobia, and candidate phylum OP3 (with no existing isolates). Microbes belonging to the bacterial phylum Acidobacteria represent one of the most diverse and commonly detected

groups of microbes in northern peatlands and other acidic wetlands (Dedysh *et al.,* 2006; Pankratov et al., 2006). Peatlands isolates from this phylum are capable of utilizing glucuronic and galacturonic acids (Dedysh, 2011). Both glucuronic acid and galacturonic acid are produced from the breakdown of *Sphagnum* mosses during decomposition (Dedysh, 2011). Species of the Acidobacteria isolated from peatlands are also capable of breaking down polymers derived from peatland plants, including pectin, xylan, lichenan and starch (Dedysh, 2011). Microbes that belong to the phylum Planctomycetes are one also of the most abundant groups of bacteria that can be found in *Sphagnum* dominated peatlands (Dedysh *et al.,* 2006). These microbes are capable of breaking down heteropolysaccharides but not cellulose or chitin; and these bacteria, along with species of Acidobacteria found in peatlands, do not appear to be N fixers (Dedysh, 2011). Another group of peat-inhabiting microbes belong to the phylum Actinobacteria, and isolates have been shown to be capable of utilizing sugars, along with polyalcohols, organic acids and aromatic compounds, but they are not able to breakdown cellulose (Dedysh, 2011).

There are microbes that belong to different bacterial phyla such as Bacteroidetes that are not abundant in acidic peatlands (Dedysh, 2011). Some species of Bacteroidetes are capable of hydrolyzing heteropolysaccharides including xylan, pectin and laminarin, and they are capable of breaking down these polymers in cold and acidic conditions, but they are incapable of breaking down cellulose or chitin (Dedysh, 2011). Lastly, microbial species from the phylum Verrucomicrobia have been found in several peatland studies (Dedysh et al., 2006; Gupta et al., 2012; Fan et al., 2016; Azarbad et al., 2015; and Amha et al., 2015). This phylum contains heterotrophic bacteria that are found in thermal environments (Dedysh, 2011), and methanotrophic bacteria that metabolize methane into carbon dioxide and biomass (Hanson and Hanson, 1996).

The role of fungi in peatlands is not very well established, however recent evidence points towards fungi being important decomposers (though still less abundant than bacteria) in the aerobic zones of acidic peatlands (Winsborough and Basiliko 2010; Myers et al 2012; Lin et

al 2011a). Mycorrhizal associations presumably are important at least in the surface horizons of drier peatlands, given the relatively low nutrient environments for plants, and a number of important shrubs found in bogs and fens (e.g. *Chamaedaphne* sp., *Kalmia* sp. *Vaccinium* sp.) are members of the family Ericacea and associated with a unique group of facultative mycorrhizal symbionts (i.e. that can also live freely; Myers et al. 2012). Evergreen shrubs and trees like *Picea* sp. and *Larix* sp. also have known ectomycorrhizal symbionts that could play key roles in peat decomposition and protecting plants from heavy metals if present in peat. Although it is rather controversial, at least some peatland sedges have also been reported to form symbioses with arbuscular mycorrhizal fungi (Turner et al., 2000).

Archaea and bacteria involved in $CH_4$ production and consumption play an important role in peatlands by limiting atmospheric emissions (Gorham, 1991). Methanotrophic bacteria (aerobic $CH_4$ consumers) are found primarily in the aerated zones over the vertical gradient in a peatland (Dunfield et al., 2007; Hanson and Hanson, 1996). The aerated zone provides ideal conditions for methanotrophic microbes because of the presence of oxygen ($O_2$) and $CH_4$ (Hanson and Hanson, 1996). These methanotrophic bacteria include species from the phyla Proteobacteria (class Gammaproteobacteria and Alphaproteobacteria) and Verrucomicrobia (Dunfield et al., 2007; Hanson and Hanson, 1996; Op den Camp et al., 2009). Other peatland methanotrophs include a subset of Eubacteria known as methylotrophs (Hanson and Hanson, 1996). These methylotrophs utilize methane for energy production and growth (Hanson and Hanson, 1996). In contrast, methanogenic microbes (anaerobic $CH_4$ producers) thrive in the waterlogged, anoxic zones of peatlands, and include specialized Archaea (Kamal and Varma, 2008). These methanogenic Archaea include organisms from the phylogenetic orders Methanopyrales, Methanobacteriaceae, Methanococcales, Methanomicrobiales, and Methanosarcinales (Garcia et al., 2000), which thrive in the absence of oxygen (Kamal and Varma, 2008). Organisms from these phylogenetic orders are capable of producing $CH_4$ gas through two main processes, namely,

acetotrophic (producing $CH_4$ by using acetate as a substrate) and hydrogenotrophic (produce $CH_4$ using hydrogen) methanogenesis (Conrad, 1999; Whalen, 2005).

### 1.4.2 Microbial Activities: Enzymes involved in Peat Decomposition

The initial degradation of *Sphagnum* moss and other plant material occurs through fragmentation and leaching by invertebrates, while subsequent stages of tissue degradation occur primarily through microbial activity (Reddy and DeLaune, 2008). Microbes excrete enzymes that hydrolyze complex plant tissues and release monomers, which then undergo catabolism in either the aerobic reactions in the acrotelm or anaerobic reactions in the catotelm; $O_2$ or alternative electron acceptors or fermentation processes then play a role oxidizing this dissolved organic matter (Reddy and DeLaune, 2008).

The degradation of *Sphagnum* mosses and other vascular tissues is controlled by several suites of enzymes, including per-oxidase, phenol-oxidase, N-acetyl-glucosaminidase (glucosaminidase), β-glucosidase (glucosidase), phosphatase, and arylsulphatase. Per-oxidase and phenol-oxidase are both lignase enzymes and they both contribute to the degradation of lignin and other complex polymers (Romanowicz *et al.,* 2015). Extracellular lignin and Mn per-oxidases are two per-oxidases that have been argued to play a role in lignin and possibly phenolic degradation (Sinsabaugh, 2009b). It has also been argued that oxidative stress (Brown *et al.,* 2007), the presence of phenolic compounds (Sinsabaugh, 2009b), and watertable level (Romanowicz *et al.,* 2015) are factors that induce the activity of per-oxidase in microbes.

Glucosidase, glucosaminidase, arylsulphatase and phosphatase are also extracellular hydrolytic enzymes that are produced by bacteria and fungi, and are responsible for plant litter and subsequent peat decomposition (Marx *et al.,* 2001). These hydrolases are key in determining decomposition and interrelated nutrient cycling in complex organic substrates like in peatlands (Marx *et al.,* 2001). Firstly, glucosidase enzyme catalyzes the breakdown of glycosidic linkages between carbohydrates and other molecules, which releases smaller sugars (Deng and Popova

2011). This enzyme supports microbial life in soil by providing important labile carbon and energy, and notably it is largely responsible for the breakdown of cellulose derived from plants (Deng and Popova 2011). Secondly, chitin (mainly fungal in origin) and other polymers containing β-(1,4)-N-acetyl-D-glucosamine (NAG) units make up a significant fraction of the humus-bound nitrogen in wetland soils (Kang *et al.,* 2005). These NAG unit polymers are hydrolyzed by glucosaminidase enzymes, and as such, their decomposition is important in the nitrogen cycle in wetlands (Kang *et al.,* 2005). Thirdly, arylsulphatase is an important hydrolytic enzyme because of its role in the soil sulphur cycle, releasing sulphate ions from arylsulphate esters including sulphated polysaccharides and phenolic sulphate (Press *et al.,* 1985, Oshrain and Wiebe 1979). Finally, phosphatase is also an important microbial extracellular enzyme, because it is responsible for removing the phosphate group from organic materials in the dephosphorylation process (Turner *et al.,* 2002). This dephosphorylation occurs when phosphatase hydrolyses phosphoric acid monoesters into phosphate ion and a free hydroxyl group (Turner *et al.,* 2002).

### 1.4.3 Microbial-Mediated Greenhouse Gas Fluxes in Peatlands

Northern peatlands act as persistent C sinks for atmospheric $CO_2$, with a unique ability to sequester and store extremely large amounts of C in their thick organic soils (Gorham, 1991); this is due to the net primary production in the peatland being far greater than the decomposition of organic matter by microorganisms (Moore and Bellamy, 1974; Clymo, 1984). However one of the consequences of slow, anaerobic decomposition of organic materials in the catotelm includes the production of $CH_4$; and in wetter sites, large amounts of $CH_4$ diffuses through the acrotelm (before aerobic $CH_4$ oxidizers can act) and then to the atmosphere making these peatlands a net $CH_4$ source (Matthews and Fung, 1987; Barlett and Harris, 1993; Hein *et al.,* 1997; Panikov, 1999; Smith *et al.,* 2004; Basiliko *et al.,* 2004). Peatlands contribute just over $1/10^{th}$ of the world's $CH_4$ emissions (Wahlen, 1993), which is a staggering amount because they occupy such a

small percent of the earth's landmass. Microorganisms are responsible for peat decomposition and net $CO_2$ release, as well as both $CH_4$ production and oxidation (where the combined effect influences $CH_4$ emissions). Although research has related broad environmental (watertable position, temperature) and plant community controls on greenhouse gas emissions from peatlands, the role of microorganisms is perhaps more poorly understood. This is in spite of the fact that the diversity of microbial communities in peatlands has been explored for some time (Begak, 1926; Waksman and Purvis, 1932; Williams and Crawford, 1983); in particular there are still large gaps in our understanding in this regard, including how communities will respond to human-related environmental changes (Andersen et al. 2013a). Microbial communities play a large role in peatland ecosystems by controlling the organic C turnover in the peat that includes the production and cycling of greenhouse gases.

## 1.5 Metals in Northern Peatlands

The boreal biome holds the largest number of undisturbed peatlands, however a moderate area of these wetlands in both Canada and northern Europe and Asia have been exposed to elevated sulphur (S) and/or metal contamination (Wells *et al.,* 2011). This type of contamination is particularly seen in peatlands that are in close proximity to mining and smelting of sulfidic ores, and metal contamination in peatlands has been documented near smelting operations throughout Ontario and around the world (Gignac and Beckett, 1986; Taylor and Crowder, 1983). Sudbury, Ontario, Canada is a well-known nickel and copper mining community, that has had over a century of emissions resulting from smelting which led to increased deposition of metals, including Fe, Pb, Cu, Ni, Co, Zn, Cr, and Cd (Adamo *et al.,* 2002; Hazlett *et al.,* 1984; Hutchinson and Whitby, 1977). Copper and nickel ore was mined in Sudbury as early as the 1880s using open-air roast bed that led to severe localized environmental contamination in the region, including S, acid and heavy metal deposition (Freedman and Hutchinson, 1980a; Freedman and Hutchison, 1980b; and Hutchinson and Whitby, 1974; Winterhalder, 1996). In

later years, newer technology saw the use of stacks to disperse waste (metals and gases) into the atmosphere, causing the widespread acidification and degradation of both terrestrial and aquatic ecosystems in the Sudbury region, which led to the area becoming severely damaged and barren (Gignac and Beckett, 1986; Gunn 1995; Amiro and Courtin, 1981; Keller *et al.,* 2007). There have been many strategies that were implemented since the 1970s to reduce the devastation of the mining and smelting industry, including the construction of the world's largest smokestack at the Copper Cliff complex, in an attempt the dilute the waste emission from mining (Gunn *et al.,* 1995). This super-stack in addition with government mandates and newer technologies, has allowed for the reduction of S and heavy metal emission; which in turn, allowed for chemical recovering of the land and lakes and re-greening and restoration of plant species in the region (Gunn *et al.,* 1995; Keller *et al.,* 2007; Szkokan-Emilson *et al.,* 2011; Szkokan-Emilson *et al.,* 2013). Fortunately, there have been major reductions in the emission of pollutants in the Sudbury region since the 1970's, with an about > 95% decrease in atmospheric emissions.

There have been many studies conducted in the region monitoring the effects of mining, and the direction of recovery over time. Studies have been focused on acid and metal impact, ecosystem recovery, and on the reduction of greenhouse gas emissions (Adamo *et al.,* 2002; Hazlett *et al.,* 1984; Hutchinson and Whitby, 1977). Nevertheless, Sudbury has had over a century of pollution that continues to have an effect and cannot be erased. Soils and surface waters still remain contaminated in several areas (Meadows and Watmough, 2012; Keller *et al.,* 2007; Nriagu *et al.,* 1998), and most of the historic deposition remains stored in the peatlands throughout the area (Gignac and Beckett, 1986; Taylor and Crowder, 1983).

There is a range of poor fens that exist in the northern landscapes that have inflow of water that originated from base-poor rocks, which causes these fens to be highly acidic like bogs because there is less anion influence to raise the pH (Bedford *et al.,* 1999). These poor fens can also be found in the Sudbury area with pH ranging from 4.0 to 5.5, caused by low base influence,

minerotrophic inputs and drainage, precipitation, and atmospheric anthropogenic particle inputs (Bedford *et al.,* 1999).

High-oxidized S, Ni and Cu deposition can have a range of impacts in peatlands. In the Sudbury region, it has been documented that Sphagnum mosses have been largely eliminated while ericaceous shrubs have become more dominant and the resulting surface peats are highly humified (Gignac and Beckett 1986). Regarding direct and indirect effects on peatland microbial communities, shifting plant communities would inevitably influence rates of litter and peat decomposition (particularly when antimicrobial constraints are lifted as Sphagnum disappears), high S loading could stimulate anaerobic respiration and further speeding peat humification and mineralization, and finally the roles of Ni and Cu can be both toxicological (causing oxidative stress) or nutritional (e.g. required for certain enzyme systems in aerobic and anaerobic C cycling; Murrell et al. 2000; Basiliko and Yavitt 2001). Luke et al. (2015) carried out one of the first studies on smelting deposition impacts on peatland microbial communities in the Sudbury region that found distinct microbial communities along a metal gradient; which was driven by metal concentration, peat humification and substrate quality. However, their study did not examine the effects of the Sudbury smelting legacy on peatland microorganisms through Illumina MiSeq microbial profiling, nor did it examine extracellular enzymatic reactions or in situ microbially mediated gas fluxes.

## 1.6 Study Objectives and Significance

Despite the improvement to mining practices and major reductions in emissions over the years, high metal concentrations, and the legacy effects of sulfate deposition still persist. There are over 33,000 ha of wetlands in Sudbury, with the majority of these being peatlands, and many of these wetlands and peatlands are directly connected to the 47,000 ha of lakes, streams and rivers in the area (Monet, 2013). Sudbury provides a unique environment in which to study the effects of metal and S contamination in peatlands, and more specifically, how the legacy of this

region has affected the microbiology of these sites. This study is necessary to identify any links that may exist between peatland microbial diversity and community structure and function, as well as how industrial activities have affected these dynamic relationships. This will assist in understanding how past and future smelting activities throughout the world affect peatland functioning and feedbacks, including in areas such as The Ring of Fire in Ontario's Far North and in many regions of China and Russia.

This thesis addresses the following main research questions: 1) In what ways has the smelting legacy in Sudbury impacted the microbial community structure and phylogenetic diversity in peatlands across sites (richer to poorer, and low to high pollution deposition) and peat depths; 2) how has the metal and S deposition legacy of the area affected microbial functioning, particularly to extracellular enzymatic reactions and the net emissions of the greenhouse gases $CH_4$ and $CO_2$; 3) Across this study and gradients, how does microbial community structure link to biochemical/biogeochemical functioning? To answer these questions I characterized the microbial community structure, diversity and function in surface and deeper peat horizons in peatlands along a metal contamination gradient in Sudbury, Ontario.

# 2 Methods

## 2.1 Study area

Sudbury, Ontario (46° 29' 24" N, 081° 00' 36" W) is situated within the Precambrian shield in Northeastern Ontario, Canada. This area has the largest nickel-copper (Ni-Cu) sulphide ore bodies known on Earth, which are accessible because of a large impact crater and subsequent Pleistocene glacial advances and retreat (Adamo *et al*., 1996). Numerous Cu and Ni mines and 3 large smelting complexes have been established over the last century to extract these minerals. This has led to the pollution of the area primarily with $H_2SO_4$, $Ni^{2+}$ and $Cu^{2+}$, including of local peatlands, which make up between 10-20% of the land surface. Eleven poor to intermediate fen sites were chosen across a pollution gradient in Sudbury to determine the impacts of Cu and Ni smelting. These sites were selected based on their distance from the Copper Cliff, Falconbridge, and Coniston industrial smelting operations; with seven of the sites being situated under 25 km away from the smelters and the remaining four sites being situated under 55 km away from the smelters. All of the selected sites have had some history of disturbance from acid deposition and metal contamination, except perhaps for two sites (Cartier1 and Cartier2) located upwind 55 km from the nearest smelter. These two sites were chosen as reference sites because they were the least impacted by the impact of the smelters based on peat and porewater chemistry and the presence of typical poor fen vegetation communities. The locations and summary characteristics of the eleven peatlands can be found in Appendix 7.3: Table 7.3, Table 7.4 and Table 7.5.

Six of the study sites were situated south of the city center, three sites were located north and northeast of the city center, and the remaining two site was located northwest of the city center (Figure 2.1). The wind direction for aerial deposition predominantly travels from west to east (Semkin and Kramer, 1976), falling on the seven sites directly under the smelters first before taller smokestacks were built, then traveling over the two sites that were north and northeast of

the smelter. This means that Cartier1 (treed) and Cartier2 (lawn) were outside of the main deposition zone of pollutants. One of the main impacts of the prolonged S and metal loading has been shifts in plant communities, notably with the most polluted sites having lost all or nearly all of their *Sphagnum* mosses that otherwise would presumably have been present and/or dominant based on local site topography, hydrological characteristics and peat chemical properties. Thus, although definitive site classification was tricky, sites appeared to be poor to intermediate fens dominated by sedges and/or shrubs including *Eriophorum* sp., *Camaedaphne* sp., *Carex* sp. and *Ledum* sp. (Figure 2.2). Certain sites (notably the 2 reference sites) also had up to 3 species of *Sphagnum* mosses in a continuous understory mat (Figure 2.2).

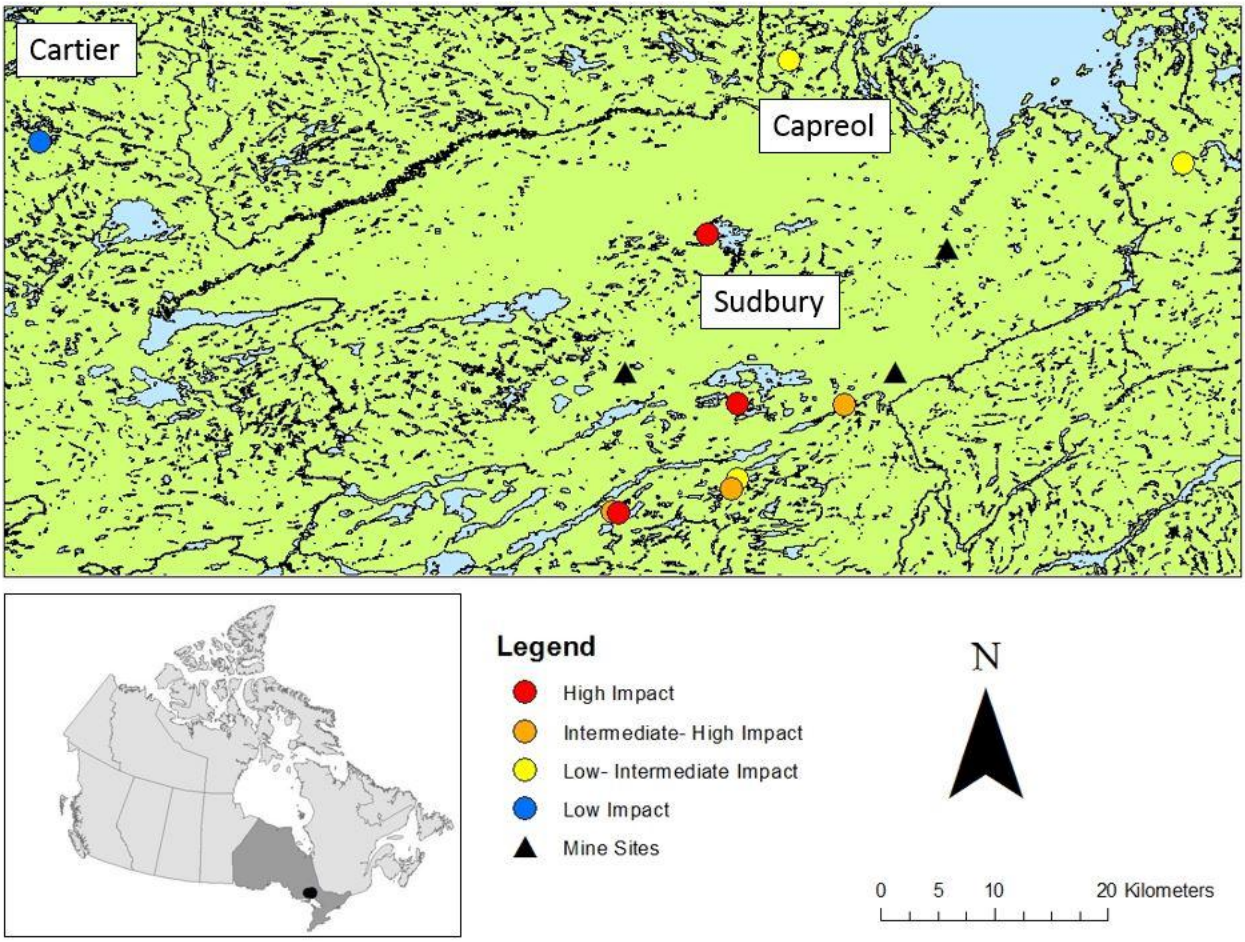



Figure 2.1– Map of Canada showing the location of Sudbury, Ontario; location of the eleven sites studied and the three smelters, namely, Copper Cliff, Coniston, and Falconbridge smelters in Sudbury, Ontario, Canada. Sites colored in green represents the low-impacted sites, blue represent the intermediate-low impacted sites, yellow represents the intermediate-high impacted sites, red represents the high-impacted sites, and purple/black triangles represent the smelters.

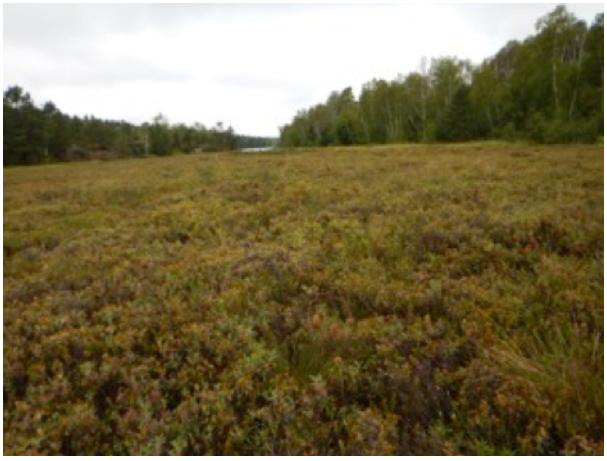
A

B

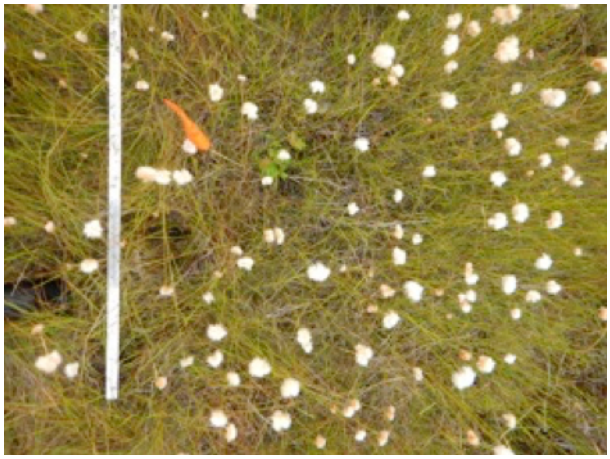
C

D

E



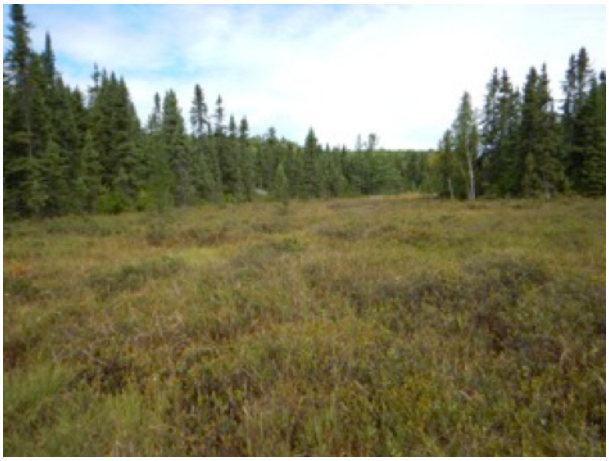
F



G

H

I



J

K

**Figure 2.2-** Photos of the eleven peatlands selected for this thesis over the 2014 field season (A: Ashigami, B: Broder, C: Cartier1 (treed), D: Cartier2 (lawn), E: Clearwater, F: Crowley, G: Daisy I, H: Laurentian, I: Long Lake, J: Rockcut and K: Whitson).

## 2.2 Field sampling

Site assessment and peat sampling were conducted during June-August 2014. Each site was divided into three plots along a 60 meter transect near the center of the peatlands to avoid shallower peat deposits and transitional plant communities along the edges that were not typically of the broader sites. Presumably this also would reduce impacts to microbial communities and peat chemistry that may be different closer to the edge due to road salts and other pollutants. I sampled four depths (0-10 cm, 10-20 cm, 30-40 cm, and 60-70 cm) at each sampling plot (sample replicates), yielding 12 samples per site for subsequent characterization of chemical and physical properties, microbial biodiversity and community structure, and microbial activity (extracellular enzyme activities) described below.

At each site, 12 peat samples were collected using a Russian Corer and a meter stick. The fresh peat samples were used for von Post classification (Appendix 7.1: Table 7.1) (von Post, 1922), temperature (degrees Celsius), and moisture (Appendix 7.2: Table 7.2). This information provided important context for the physical and chemical microbial environments (Appendix 7.3: Table 7.5). Approximately 100 g of peat was then collected from each depth at each plot and placed in sterile Whirl-Pak bags and were frozen immediately for DNA, enzyme and chemical analyses. Collecting samples at 10 cm depth increments allowed for the analysis of past environmental impacts versus present-day environmental impacts, such as mining effects (further studies looking at 1-cm depth increments would have to be conducted in order to determine yearly changes in mining effects and other environmental changes).

The vascular vegetation in each plot at each site was identified to the species nomenclature using field guides and recorded, while richness (but not species ID) was determined visually for *Sphagnum* sp. and mosses. Percent cover of each plant species (or collective Sphagnum species) was determined by visual estimation of all plants within the plots. Pore water from each plot was collected after coring was completed. The corer removed

approximately a 4 cm diameter and 70 cm deep peat section from each plot and this allowed for the pore water to fill into the area that was cored. Pore water was collected using 50-mL Falcon tubes. The pore water was analyzed for conductivity and pH using a conductivity and pH meter (Hanna Instruments HI991002).

Of the 11-peatland sites that were studied for peatland microbial diversity and function during the 2014 field season, eight of these sites were selected in order to study in situ gas fluxes. These sites represented the range of surface peat, vegetation, hydrology and biogeochemistry observed throughout the regional survey, with sites at the low and high-ends of the metal concentrations observed due to pollution. The high-impacted sites were dominated by shrubs and included Laurentian, Whitson and Clearwater (Appendix 7.6: Table 7.11), which were all within 14 km of the Copper Cliff Smelter. Shrubs, sedges and mosses, dominated the intermediate impacted sites, which included Broder, Ashigami and Rockcut (Appendix 7.6: Table 7.11). The low-impacted sites included Cartier1 (treed) and Cartier2 (lawn), and Sphagnum mosses dominated these sites (Appendix 7.6: Table 7.11).

Gas collection, vegetation assessment and long-term water table and temperature assessments were conducted in all eight sites during August-September 2015 field season. Permanent boardwalks were built in each site and six plots were chosen along the boardwalks where gas fluxes, vegetation, and microtopography were characterized. The six plots were distributed across the peatland on different microtopography and vegetation to ensure that a representative average gas flux rate was collected. For each replicate, one PVC collar with a 25 cm diameter was placed in the peat at a depth of 8-10 cm. A 17 L chamber wrapped with heavy-duty foil to block light was placed on each collar to collect gases using a 20-mL syringe.

Temperature and watertable position was measured using an auto-logging method (Appendix 7.9: Figure 7.15 and Figure 7.16). HOBO™ water sensors and temperature loggers were installed at 50 cm and 15 cm depths respectively from August 18th-November 4th

2015. The loggers were set to record at 15-minute intervals in order to calculate daily and monthly averages. The water sensors were placed in a 1-meter PVC pipe wells with slit openings covered with nylon mesh to allow the flow of water into the wells. HOBO™ temperature loggers were placed in all eight sites, while the HOBO™ U20 water sensors were placed in Ashigami, Cartier1, Clearwater, Rockcut and Laurentian. Understanding the fluctuation of water table allowed for gas flux data to be analyzed while taking into account the effects of the watertable position that is known to affect the relative degrees of methane production and consumption.

## 2.3 Metal Analysis

As a measure of bioavailable metals following Abedin et al. (2012), lithium nitrate extracts were prepared from each peat sample and characterized by ICP-MS and IC (Varian 810 model ICP-MS with an SPS3 auto-sampler). To accomplish this, 10 g of each sample was homogenized, weighed and dried at 55°C for 48 hours until all the moisture was removed from the samples. 1 g of dried sample was mixed with a 40 mL 0.01M $LiNO_3$ (0.6895 g/L) solution in a 50 mL falcon tube. The 1:39 ratio of sample to 0.01M $LiNO_3$ was used because the samples were organic/peat samples, which required more 0.01M $LiNO_3$ solution than usual as dried peat absorbed a lot of the solution without leaving enough free liquid to be used further in the procedures. The falcon tubes containing the mixtures were placed on their sides on a shaker at 150 RPM to shake for 48 hours. The samples were then placed in a centrifuge at 8,000 RPM for 5 minutes and filtered with a .45-micron filter paper to obtain 12 mL of supernatant sample. Two blank samples were made from shaking 0.01M $LiNO_3$ solutions in order to account for any potential contaminants in the reagent, tubes or laboratory water.

1:10 dilutions of the solutions were performed (1 mL sample + 9 mL ultrapure water) and loaded into racks for the instrument auto-sampler. Multi-element standards were used from Inorganic Ventures to make a series of calibration standards from 0.5 ppb to 1000 ppb with a few elements (Na, Mg, Ca, K, Fe, Al, P) covered to 25000 ppb (since elements are generally found

higher). Additionally, a 1-ppm solution of Rhenium (Re) and Ruthenium (Ru) was used as an internal standard, which gets mixed with each sample to measure and correct for fluctuations in the instrument's performance (Re correcting the heavier elements and Ru the lighter ones). The primary data were corrected for the 1:10 dilution of sample with ultrapure water and the 1:40 dilution of sample with 0.01M $LiNO_3$ and was expressed per gram of dry peat.

The pH of the 1:39 sample ratio of 0.01M $LiNO_3$ was calculated for each sample and recorded to be used to further categorize the samples by site replicates and depths. The pH values by both site and depth increments corresponded closely, therefore these values were not converted from pH to the logarithmic mean. Instead, the pH values were kept as mathematical means for all samples by site and depth increments. The elemental or ion concentrations were analyzed concomitantly with the pH, enzyme and community structure data to observe possible linkages between these variables (Appendix 7.4: Table 7.6-7.10, Figure 7.1).

## 2.4 Microbial Community Analyses

16S rRNA genes from bacteria and archaea were analyzed at the US DOE-JGI facility via the "Global Peatland Micro-biome Project" (2013), for depths ranging from 10-20 cm, 30-40 cm and 60-70 cm. This included high throughput sequencing using the Illumina MiSeq platform to observe related community structures and phylogeny. Duk (version 1.05) was used to filter out contaminants, and PCR primers of the conserved region were trimmed away and for paired-end reads, both the forward and the reverse primers were found (or the entire pair was filtered). The reads were trimmed as a pair and the last base from whichever end had the highest expected error in a window 5bp wide was removed. The reads were trimmed from mean + 3 standard deviations to the mean -3 standard deviations in 0.5 standard deviation steps. After each trimming step, the pairs were merged into single sequences with Pandaseq 2.5 (Masella et al., 2012). The pairs that were not merged continued to the next round of trimming. The paired reads that were not

combined were discarded. Finally, the merged reads were filtered if they had an expected number of errors that exceeded the threshold.

Filtered sequences were analyzed in QIIME (Caporaso et al., 2010) using the USEARCH software (v7.0.959i86linux32) to cluster OTUs, and using the RDP Classifier (Wang et al., 2007) for taxonomic classification of the resultant cluster centroid sequences. The saved output files were merged, de-replicated, and stored by decreasing abundance using QIIME. Low abundance sequences were separated and excluded from clustering, although they were mapped and counted later in the procedure. Clustering was done iteratively starting at 99% identity, and then decreasing by 1% identity until the level described in the configuration file was obtained. This gave 97% for the 16S sequences. Rare sequences that could not form their own clusters were mapped to the cluster centroid sequences and counted. Centroid sequences were compared to the reference database and the likely chimeric sequences discarded using UCHIME. Operational taxonomic units (OTUs) were assigned using the Greengenes database using 97% similarity (DeSantis et al., 2006) and verified using the RDP Classifier (Wang et al., 2007). Sequence data were initially processed using QIIME and then further examined using multivariate analysis for related community structures in R and phylogenetic analysis in MEGA.

## 2.5 Enzyme Analysis

The activity of microbial hydrolases and lignases in the peat samples were determined using published protocols (Sinsabaugh, 2009a; Dunn et al., 2014). For all enzyme assays, 0.50 g of each sample was homogenized in 60 mL of 50-mM acetate buffer (pH = 5.0) (Appendix 7.8: Table 7.15-Table 7.17 and Figure 7.14).

### 2.5.1 Lignase Assays

Firstly, a lignase assay test was used to analyze phenol-oxidase and per-oxidase activity in each peat sample. 200 µL of sample was aliquotted into 50 mM of acetate buffer (pH = 5.0),

25 mM L-DOPA substrate and 0.3% $H_2O_2$ in the micro-plate wells and incubated for 4 hours. The absorbance was determined spectrophotometrically at 460 nm for 5 minutes (Biotek Synergy H1 Micro-plate Reader). The enzyme activity for each of the samples was calculated using the extinction coefficient of L-DOPA with horseradish peroxidase, [Extinction coefficient (EC) = OD/c*l, where OD= peak absorbance, c= concentration (M) of LDOPA and l= length of light path] (Appendix 7.8: Figure 7.14, Table 7.16).

### 2.5.2 Hydrolase Assays

A hydrolase assay was used to analyze microbial activity for phosphatase, β-D-glucosidase, arylsulphatase, and N-acetyl-β-D-glucosaminidase in the peat samples (Appendix 7.8: Table 7.15 and Table 7.17). Phosphatase is an enzyme that allows for the removal of a phosphate group from an organic molecule through hydrolysis of phosphoric acid monoesters releasing a phosphate ion (Turner et al., 2002). Phosphatase activity relates to the ability of the microbial community in the peat samples to mineralize and access phosphorus (P). 200 µL of homogenized peat sample was suspended in 50 mM of acetate buffer (pH=5), 10 µM 4-methylumbelliferone (MUB), and 200 µM MUB-phosphate in a micro-plate and incubated for 2 hours. The absorbance was determined spectrophotometrically at an excitation of 365nm and an emission wavelength of 450nm for 5 minutes (Biotek Synergy H1 Micro-plate Reader).

β-D-glucosidase is an enzyme that catalyzes the hydrolysis of glycosidic linkages that form between molecules of carbohydrate and results in smaller sugars (monomers) being release, for example, glucose (Deng and Popova, 2011). 200 µL of homogenized peat sample was suspended in 50 mM of acetate buffer (pH=5), 10 µM 4-methylumbelliferone (MUB), and 200 µM MUB-B-D-glucopyranoside in a micro-plate and incubated for 2 hours. The absorbance was determined spectrophotometrically (excitation of 365nm, and emission wavelength of 450nm for 5 minutes).

Arylsulphatase is an enzyme that catalyzes the removal of sulphate ion from compounds that fall into the arylsulphatase ester group (Klose et al., 2011). 200 µL of homogenized peat was

suspended in 50 mM of acetate buffer (pH=5), 10 μM 4-methylumbelliferone (MUB), and 200 μM MUB-sulphate potassium, similar to the phosphatase test. The plate was incubated for 2 hours and placed into the spectrophotometer for 5 minutes.

Lastly, N-acetyl-β-D-glucosaminidase is an enzyme that breaks down chitin polymers. Chitin polymers are a major fraction of humus-bound nitrogen and this assay gives a picture of how active the microbial communities found in the peat soil samples were regarding accessing the key nutrient nitrogen (Kang et al., 2005). 200 μL of homogenized peat sample was suspended in 50 mM of acetate buffer (pH=5), 10 μM 4-methylumbelliferone (MUB), and 200 μM MUB-N-acetyl-β-D-glucosaminidase similar as with the phosphatase test. The plate was incubated for 2 hours and placed into the spectrophotometer for 5 minutes.

## 2.6 In Situ Gas Fluxes

Gas flux chambers were made using the top portion of Culligan® water bottles, heavy-duty foil paper, tubing, and rubber stoppers. Permanent plastic collars capable of receiving the chambers were placed six at each site, with the chambers on either side of the boardwalks. The boardwalks were built out of cedar for this study to minimize the addition of chemicals such as sulphur-based chemicals that are present in pressure-treated wood, which would add pollutants to sites that were being studied for pollution effects. These boardwalks also provided a platform to collect gas samples; this reduced the occurrence gas ebullition that would take place from direct interaction with the peat surface.

Gas sampling was conducted twice over a 1-2 month period at each site beginning in August 2015 and ending in September 2015. 20 mL syringes were used to collect four gas samples from each chamber over a 60-minute period, at a 20-minute time intervals from time zero (atmospheric) to time 3 (60 minutes). Gas chromatography was used to analyze $CO_2$ and $CH_4$ concentrations and net flux rates of each site were calculated.

## 2.7 Statistical Analysis

Statistical analyses of microbial data were conducted using R (R Core Team (2014), version 3.1.1) along with phyloseq (McMurdie and Holmes (2013), version 1.10.0), and MEGA (Kumar, Stecher, and Tamura (2015), version 7.0) to make maximum likelihood trees. Statistical analyses on the environmental data, gas data and enzyme data sets were conducted using SPSS (IBM Corp. 2012, version 21.0). For the environmental data, Pearson's correlation in SPSS was used to analyze environmental variables including copper, nickel, aluminum, potassium, calcium, von Post, temperature, moisture, and pH against distances from the Copper Cliff smelter for each of the 11 sites. These variables were then compared against each other to determine if there were any relationships between site variables. Additionally, the mean Cu and Ni concentrations were plotted against the study sites showing the respective Pearson's correlations for each metal, and von Post scores were plotted showing depths using the mean Cu and Ni concentrations.

For the microbial data set, a total of 13244 taxa were obtained after quality filtering in Qiime for singletons and chimeras. Of the 13244, 9166 OTUs (abundant and rare OTUs) were obtained after filtering out OTUs that were unclassified at the kingdom and phylum levels. Using these 9166 OTUs, three PCoA ordinations were created in the phyloseq package and plotted using ggplot2 in R (Wickham (2009), version 1.0.1). The PCoA's looked at microbial community distribution for the unweighted UniFrac distances of each OTU by site, depth and chemical status from low-impacted to high-impacted. Additionally, these 9166 OTUs were used to calculate Shannon diversity indices in the phyloseq package and plotted using ggplot2 in R (Wickham (2009), version 1.0.1) for data across the 11 sites and the three depths.

The sequencing data were transformed from absolute abundance (Appendix 7.7) to relative abundance for all OTUs and those OTUs that had > 1% relative abundances by site, were selected for downstream analyses (40.1% of total data set). Principal coordinate analysis (PCA) was conducted using the vegan package (Oksanen (2015), version 2.2-1) on the > 1% dataset for

all sites and depths and for individual depths by site, to determine the effects of environmental properties on microorganisms. The percent abundance of each of the 45 OTUs (relative abundance > 1% for each site) was analyzed using phyloseq and plotted using ggplot2 in R (Wickham (2009), version 1.0.1). Indicator species analyses were conducted using the labdsv package in R (Roberts (2015), version 1.7-0) with 1000 permutations. The OTUs that were significantly associated with site, depth or impact ($p$-value < 0.05) were considered an indicator species. Heat maps were made using the Heatplus package (Ploner (2014), version 2.12.0) on the > 1% dataset, and the OTUs were shown at the order level, where the Bray-Curtis distances in the vegan package (Oksanen (2015), version 2.2-1) was used for hierarchal clustering of the OTUs by sites and taxa (Appendix 7.7: Figure 7.3-713). The gplots package in R was used to plot the heatmaps (Warnes (2015), version 2.17.0). Permutational multivariate analysis (PERMANOVA) was conducted on the > 1% data sets using the RVAideMemoire package in R (Hervé (2015), version 0.9-45-2) for the data set with 49 permutations across the sites, depths and impact gradient. Additionally, PERMANOVA was conducted on the > 1% data sets using the RVAideMemoire package in R (Hervé (2015), version 0.9-45-2) for the data set across the chemical and physical variables (copper, nickel, aluminum, potassium, calcium, von Post, temperature, moisture, and pH) with 999 permutations. In all cases for the PERMANOVAs, Bray-Curtis distances in the vegan package (Oksanen (2015), version 2.2-1) were used to calculate the distance matrices.

The data set was further pruned in phyloseq from > 1% to > 2% relative abundance for each OTU at each site plus the indicator species (27.8% of total data set) to obtain 21 OTUs. This was done in order to briefly look at correlations between species and environmental factors. Univariate permutational analysis (PERANOVA) was conducted on the key 21 OTUs with 499 permutations using the RVAideMemoire package in R (Hervé M., (2015), version 0.9-45-2) and the results were plotted in a table.

The percent cover for the plant species studied was transformed using Hellinger transformation in vegan, then this was plotted against the environmental data set using the vegan package (Oksanen (2015), version 2.2-1) for redundancy analysis (RDA). A Mantel test was conducted through the ade4 package (Dray and Dufour (2007), version 1.7-2) on the plant and environmental datasets, where both datasets were converted to Euclidean distance matrices using the vegdist function in the vegan package (Oksanen (2015), version 2.2-1), and analyzed using 9999 permutations. Additionally, the plant cover data set and the > 2% OTU data set were analyzed using RDA in vegan (Oksanen (2015), version 2.2-1), where the plant cover data were transformed using Hellinger transformation in vegan and plotted against the OTU data set. A Mantel test was conducted through the ade4 package (Dray and Dufour (2007), version 1.7-2) on the plant and > 2% OTU data sets, where both data sets were converted to Euclidean distance matrices using the vegdist function in the vegan package (Oksanen (2015), version 2.2-1), and analyzed using 9999 permutations. The Mantel test estimated the correlation between the two Euclidean matrices and the *p*-value obtained was used to represent the significance of the Mantel regression coefficients from 0 following the 9999 permutations. Lastly, *Sphagnum* species richness was plotted against site, with a Pearson correlation to determine the *p*-value.

For the enzyme analyses, SPSS was used to perform ANOVA and SNK Post-hoc tests to determine significance of the data by site or by depth. The results of the ANOVA was plotted using letters on mean enzyme plots in order to show significant differences from the ANOVAs and also the mean rate of activity for each enzyme. For the enzyme data by the four depths, the ANOVA and Post-hoc tests were conducted on the log-transformed data sets because the data were not normally distributed by depth due to the magnitude of difference in the enzyme activity from the surface depth to the deep peat depths. Lastly, for the gas data set, multivariate repeated measures analysis was conducted in SPSS using Pillai's Trace and Greenhouse-Geisser for between subject and within subject effects respectively on the $CO_2$ and $CH_4$ effluxes over two collection times and over a distance gradient of metal impact.

# 3 Results

## 3.1 Chemical and Physical Characteristics of Studied Peatlands

### 3.1.1 Chemical and Physical Characteristics of Peat across a Horizontal Gradient

The 11-peatlands were grouped into four different categories, namely, "low-impacted", "intermediate-low-impacted", "intermediate-high-impacted", and "high-impacted" sites based on the range of metal concentrations present at each site (Figure 3.1). The 11-peatland sites that were sampled showed great spatial variability in both pH (with mean pH ranging from 3.51 – 6.19 over a 0-70 cm depth), and Cu and Ni concentrations (with mean bioavailable Cu ranging from 0.1898 – 2.609 mg/kg over a 0-70 cm depth and mean bioavailable Ni ranging from 0.9921 – 8.005 mg/kg over a 0-70 cm depth) (Table 3.1). The bioavailable concentrations of elements were used in this study because they represent the fraction of element concentrations that was accessible to be used by peatland organisms such as peatland microbes. These extractable Cu and Ni concentrations can be compared to total Cu and Ni concentrations conducted in other studies such as Barrett and Watmough (2016) showing total Cu concentrations ranging from <100 to >1500 mg/kg for similar Sudbury sites, and total Ni ranging from <100 to >900 mg/kg for similar Sudbury sites. Extractable Cu and Ni were positively correlated over the chosen sites (Figure 3.2), and they were also negatively correlated with increasing distance from the Copper Cliff smelter (Table 3.2). Calcium (Ca) and potassium (K) were inversely correlated with distance from the smelter, while von Post and pH were negatively correlated (Table 3.2). Additionally, peat pH over the eleven sites was negatively correlated with Cu, and the physical properties including moisture and von Post were positively correlated with Cu, Ni, Al and K (Table 3.3). Von Post was also negatively correlated with moisture (Table 3.3). There were other significant variations in the interaction with peat chemistry and physical properties (Table 3.3), and these differences along with the significant differences in relation to distance from Copper

Cliff, reflected the characteristic differences between the various chosen sites and the four categories in which they were placed relative to the Cu concentration gradient.

The differences in site chemical and physical properties were observed using a PCA, with 58% of the variability being explained in the first two axes (Figure 3.3). The first axis (33.68% variability) represented the chemical and physical smelter deposition gradient, with positive loadings for Cu, Ni, Al, pH, von Post, moisture and temperature; and the second axis (24.37% variability) represented the physical properties studied including temperature and moisture (Table 3.4). The sites that were influenced by the chemical and physical properties in the peat included Broder, Long Lake, Daisy-I, Laurentian and Whitson (Figure 3.3). Additionally, the sites that were most affected by the physical properties of the peat, namely, moisture and temperature were Ashigami, Cartier1, Cartier2, Daisy-I, Long Lake and Rockcut (Figure 3.3). Daisy-I and Long Lake had the highest loadings along both axes and Cartier1 was clearly separated from the other sites with a negative loading on the first axis (Figure 3.3).

**Table 3.1**- Chemical properties of the eleven chosen sites over a 0-70 cm depth range, categorizing the sites from low metal impacted to high metal impacted for the five representative metals. Error is represented as standard error of the mean (SE).

| Sites | Categories | Mean pH | Mean von Post score | Mean [Al] (mg/kg) | Mean [Cu] (mg/kg) | Mean [Ni] (mg/kg) | Mean [Ca] (mg/kg) | Mean [K] (mg/kg) |
|---|---|---|---|---|---|---|---|---|
| Cartier1 | Low Impacted | 3.508 +/-0.01 | 5.083 +/- 0.56 | 9.120 +/-1.42 | 0.1898 +/-0.04 | 0.9921 +/-0.17 | 307.2 +/-32.60 | 307.9 +/-90.97 |
| Cartier2 | Low Impacted | 3.531 +/-0.03 | 4.083 +/- 0.31 | 18.61 +/-1.47 | 0.2753 +/-0.05 | 1.880 +/-0.31 | 569.7 +/-44.62 | 107.3 +/-29.61 |
| Ashigami | Intermediate-Low Impacted | 5.172 +/-0.22 | 5.250 +/-0.60 | 5.959 +/-1.05 | 0.2319 +/-0.07 | 2.577 +/-0.56 | 425.0 +/-54.78 | 144.0 +/-46.15 |
| Rockcut | Intermediate-Low Impacted | 4.312 +/-0.07 | 5.400 +/-0.60 | 37.38 +/-11.25 | 0.3875 +/-0.13 | 3.060 +/-1.03 | 105.1 +/-20.95 | 115.4 +/-33.71 |
| Broder | Intermediate-Low Impacted | 5.242 +/-0.12 | 5.750 +/-0.62 | 15.30 +/-3.49 | 0.7443 +/-0.22 | 6.711 +/-2.05 | 316.3 +/-26.27 | 167.4 +/-64.33 |
| Long Lake | Intermediate-High Impacted | 5.791 +/-0.14 | 6.167 +/-0.61 | 11.03 +/-1.82 | 0.9684 +/-0.28 | 1.567 +/-0.33 | 189.1 +/-37.46 | 70.15 +/-21.60 |
| Crowley | Intermediate-High Impacted | 4.162 +/-0.18 | 6.667 +/-0.50 | 8.871 +/-1.52 | 1.356 +/-0.43 | 4.865 +/-1.47 | 309.1 +/-34.48 | 132.9 +/-55.67 |
| Daisy I | Intermediate-High Impacted | 5.150 +/-0.24 | 6.444 +/-0.24 | 16.18 +/-5.07 | 1.609 +/-0.50 | 2.548 +/-0.45 | 45.31 +/-3.75 | 198.0 +/-77.88 |
| Laurentian | High Impacted | 4.801 +/-0.13 | 6.833 +/- 0.37 | 12.77 +/-1.85 | 2.372 +/-0.78 | 6.816 +/-2.10 | 382.3 +/-48.24 | 89.22 +/-33.70 |
| Clearwater | High Impacted | 3.743 +/-0.03 | 5.833 +/-0.46 | 21.09 +/-2.55 | 2.582 +/-0.93 | 6.478 +/-2.00 | 347.0 +/-48.93 | 215.5 +/-103.50 |
| Whitson | High Impacted | 6.189 +/-0.22 | 5.000 +/-0.50 | 36.35 +/-15.00 | 2.609 +/-0.97 | 8.005 +/-2.69 | 347.6 +/-82.24 | 181.7 +/-72.85 |

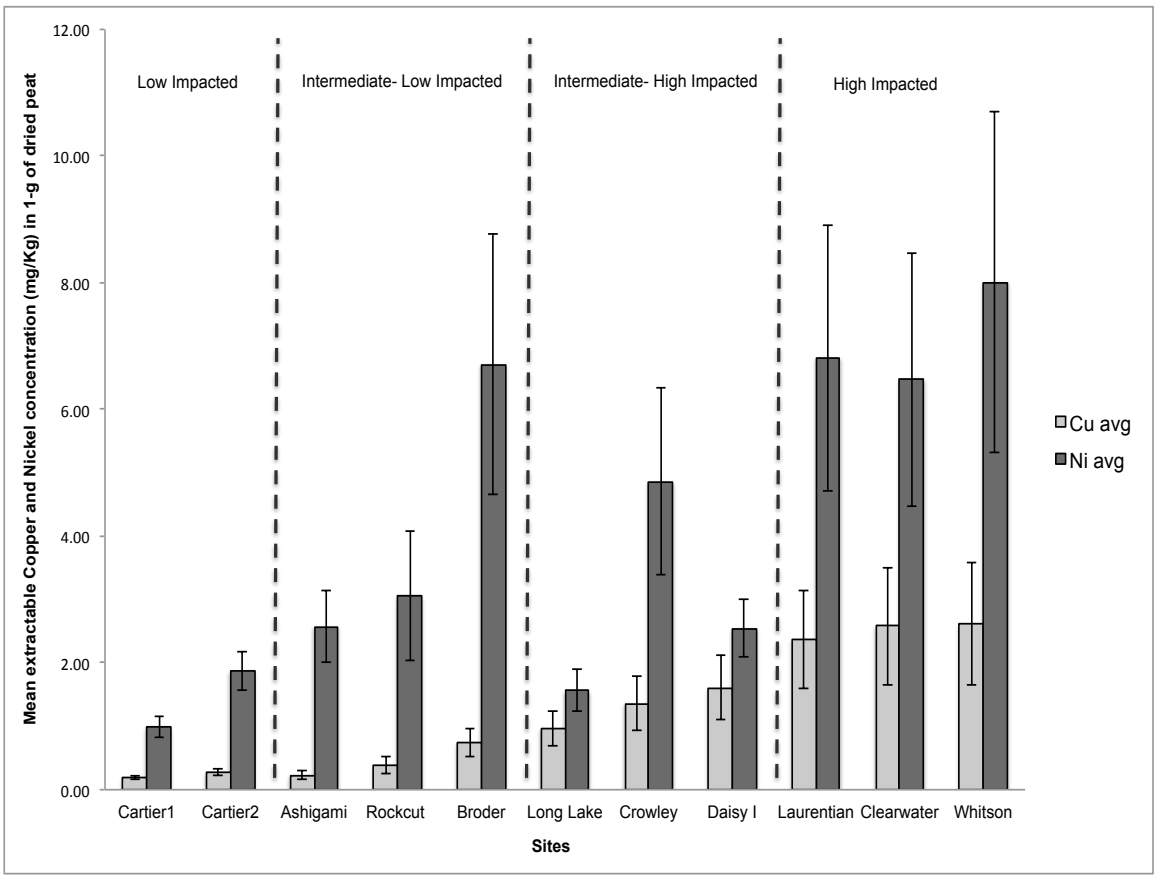



**Figure 3.3**- Mean extractable Cu and Ni concentration (mg/kg) across eleven peatland sites over a 0-70cm depth. Sites were ranked based on mean [Cu] and [Ni] from low metal impacted to high metal impacted sites. Error bars represent standard error of the mean (SE).

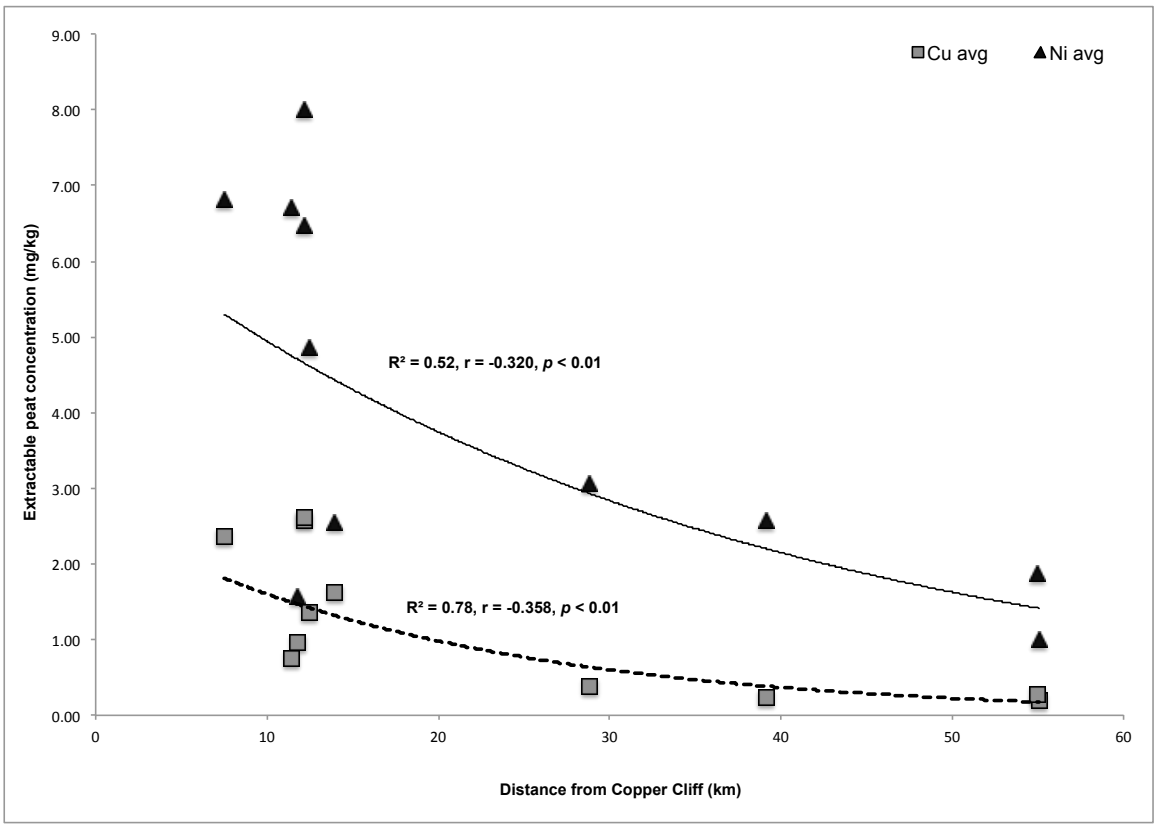



**Figure 3.4**-The relationship between Cu and Ni concentrations over a 0-70 cm depth range and the distance from the Copper Cliff Smelter in Sudbury, Ontario. The Pearson correlation is represented by r and the correlation significance is represented by *p*.

**Table 3.2**- Peat chemistry and physical properties of eleven peatland sites over a 0-70 cm depth range and the Pearson correlation (*d*) for extractable metals, pH, von Post and moisture score of the mean interactions with distance from the Copper Cliff Smelter.

| **Variables** | **Mean** | **+/- SD** | **Max** | **Min** | ***d*** |
|---|---|---|---|---|---|
| Cu mg kg $^{-1}$ | 1.19 | 0.17 | 9.58 | 0.000 | -0.358** |
| Ni mg kg $^{-1}$ | 4.12 | 0.49 | 26.2 | 0.010 | -0.320** |
| Al mg kg $^{-1}$ | 17.5 | 2.00 | 169 | 1.27 | -0.060 |
| Ca mg kg $^{-1}$ | 310 | 17.7 | 802 | 11.5 | 0.262** |
| K mg kg $^{-1}$ | 155 | 18.9 | 1.27e3 | 0.490 | 0.097 |
| pH | 4.68 | 0.09 | 7.53 | 3.18 | -0.504** |
| von Post | 5.47 | 0.19 | 9.00 | 2.00 | -0.261** |
| Moisture Score | 4.74 | 0.05 | 5.00 | 2.00 | -0.043 |

$p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

**Table 3.3**-The interaction of peat chemistry and physical properties across eleven peatland sites over a 0-70 cm depth range with the Pearson correlation (*d*) and statistical significance (*p*) for extractable metals, pH, von Post and moisture score.

| **Variables** | ***d*** | ***p*-value** |
|---|---|---|
| Cu (mg kg $^{-1}$) and Ni (mg kg $^{-1}$) | 0.806** | 0.000 |
| Al (mg kg $^{-1}$) and Cu (mg kg $^{-1}$) | 0.520** | 0.000 |
| pH and Cu (mg kg $^{-1}$) | 0.215* | 0.015 |
| von Post and Ni (mg kg $^{-1}$) | -0.268** | 0.002 |
| von Post and Cu (mg kg $^{-1}$) | -0.199* | 0.024 |
| von Post and Al (mg kg $^{-1}$) | -0.266** | 0.002 |

* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$.

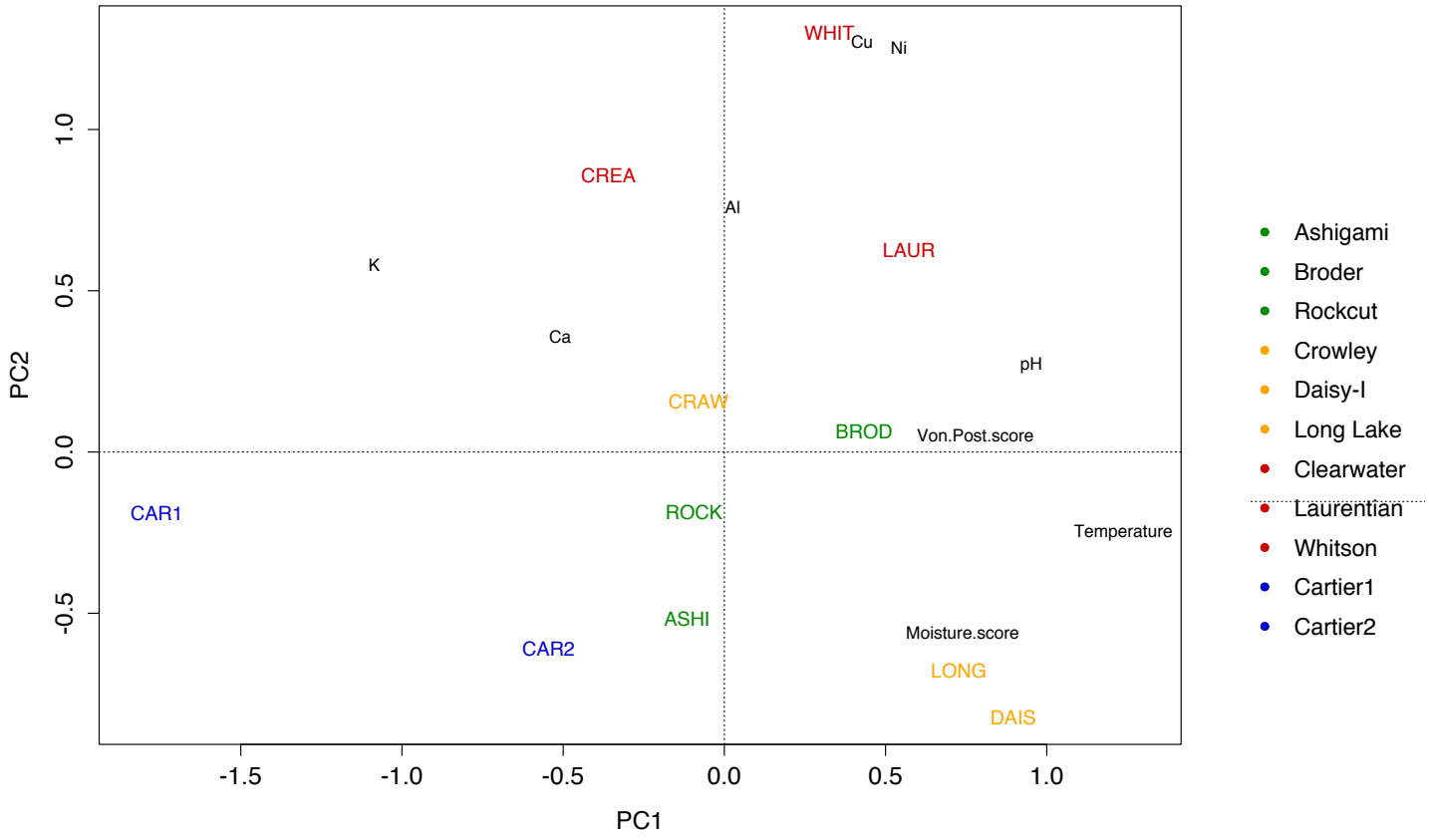



**Figure 3.5-** Principal component analysis for peat chemical and physical properties showing the variables and the peatland site loadings over a depth range from 0-70 cm. PC1 and PC2 explain 33.68% and 24.37% of the proportion of variance respectively.

**Table 3.4**- PCA factor loadings for peat chemical and physical variables over a depth range from 0-70 cm. PC1 and PC2 explain 33.68% and 24.37% of the proportion of variance of the variables respectively.

| **Variables** | **PC1** | **PC2** |
|---|---|---|
| Al | 0.01102714 | -0.35083611 |
| Cu | 0.18220717 | -0.58822589 |
| Ni | 0.23075199 | -0.57888549 |
| Ca | -0.21710209 | -0.16474781 |
| K | -0.46259841 | -0.26879498 |
| pH | 0.40573409 | -0.12380910 |
| Temperature | 0.52739958 | 0.11584698 |
| von Post score | 0.33205475 | -0.02394875 |
| Moisture score | 0.31507307 | 0.25899918 |

### 3.1.2 Chemical and Physical Characteristics of Peat over a Vertical Depth Gradient

Within each of the study sites, a decreasing trend was observed within surface samples (0-10 cm and 10-20 cm) where high-impacted sites had the highest Cu concentrations versus low-impacted sites that had the lowest Cu concentrations at the surface (Figure 3.4). Comparatively, all surface depths had much higher Cu concentrations than the medium (30-40 cm) and deep peat (60-70 cm) samples within each site (Figure 3.4). There were no trends clearly distinguishing the medium and deep peat depths across the gradient of impact, however, the least impacted sites showed higher Cu concentrations at depth compared to the other sites (Figure 3.4). With Ni, a similar decreasing trend was observed from high- to low- impacted sites, and both surface depths (0-10 cm and 10-20 cm) had much higher Ni concentrations than the medium (30-40 cm) and deep (60-70 cm) peat depths (Figure 3.5). Similarly, no trends were observed for Ni concentrations between the medium and deep peat depths (Figure 3.5).

The degree of decay measured by the von Post humification index across the eleven sites increased with depth. Across all sites, the 0-10 cm depth had the lowest von Post scores and the 60-70 cm depth had the highest von Post scores when measured along the Cu (Figure 3.6) and Ni concentration gradient (Figure 3.7). There is greater variation among the von Post scores in the deep peat samples (30-40 cm and 60-70 cm) across the sites than in the shallow samples (0-10 cm and 10-20 cm) for Cu (Figure 3.6) and Ni (Figure 3.7), however, the von Post change were highest in the surface samples for the Cu concentration gradient (Figure 3.6).

The differences in the chemical and physical properties of the peat at the four depth increments (0-10 cm, 10-20 cm, 30-40 cm, and 60-70 cm) were observed using a PCA. The two axes at the surface depths (0-10 cm and 10-20 cm) represented chemical/physical and metal/mineral gradients respectively (Table 3.5; Table 3.6); while the two axes at the deep depths (30-40 cm and the 60-70 cm) represented chemical/mineral and chemical/physical gradients

respectively (Table 3.7; Table 3.8). At the surface depth, the low-impacted and the high-impacted sites showed distinct groupings along both axes, with Cartier1 and Whitson clearly separating from the other sites (Figure 3.8). At the subsurface depth, there was overlapping observed between the low-impacted and the intermediate-low-impacted sites, and between the high-impacted and the intermediate-high impacted sites, with Cartier1 and Clearwater showing distinct separation from the other sites at this depth (Figure 3.9). Additionally, at the 30-40 cm depth, the low-impacted and intermediate low-impacted sites showed distinct groupings from the other sites, and Cartier1 and Cartier2 showed clear separation from the other nine sites, while the intermediate high and the high-impacted sites showed overlapping (Figure 3.10). Lastly, the deep peat samples showed distinct groupings for the low-impacted and high-impacted sites, and also overlapping in the intermediate impacted sites, with Cartier1 showing clear separation from the other 10 sites (Figure 3.11).

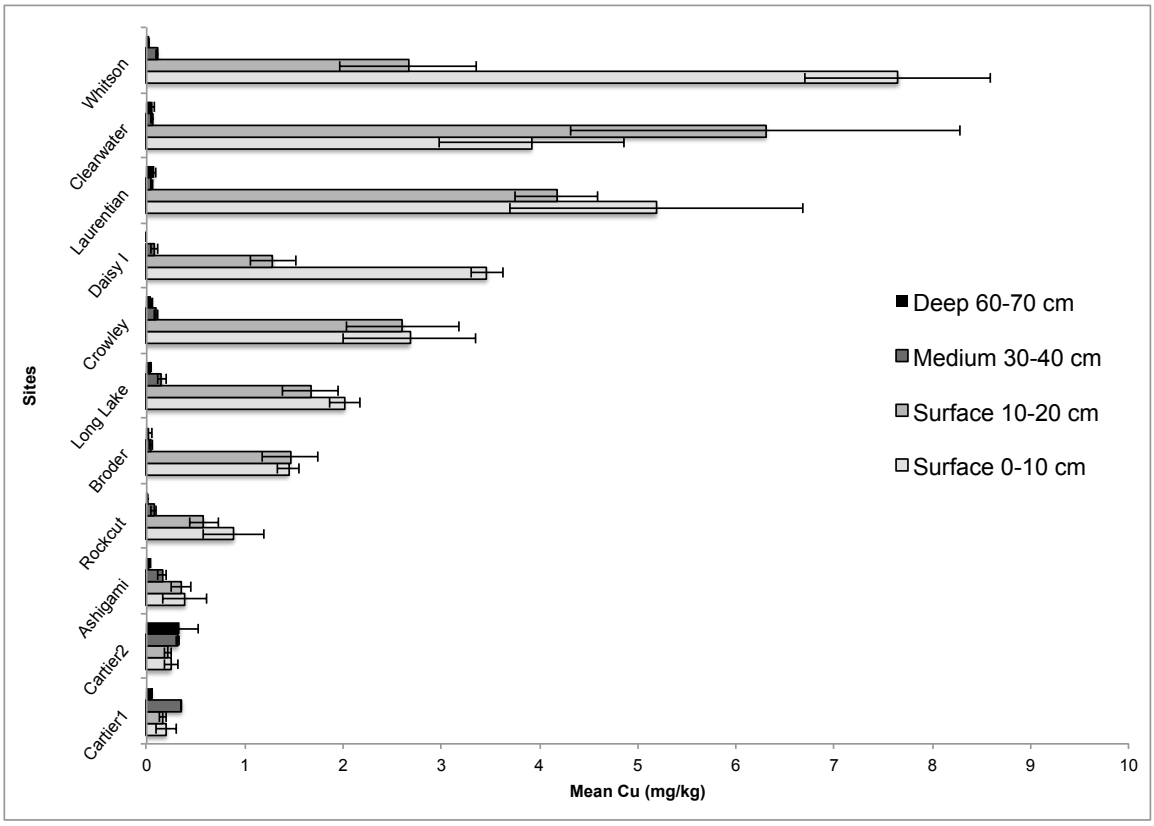



**Figure 3.6**- Peat Cu concentrations across eleven peatland sites at the surface 0-10 cm, subsurface 10-20 cm, medium 30-40 cm and deep 60-70 cm peat depths ordered over a gradient of high-impacted (top of figure) to low-impacted (bottom of figure) sites. Error bars represent SE of the mean.

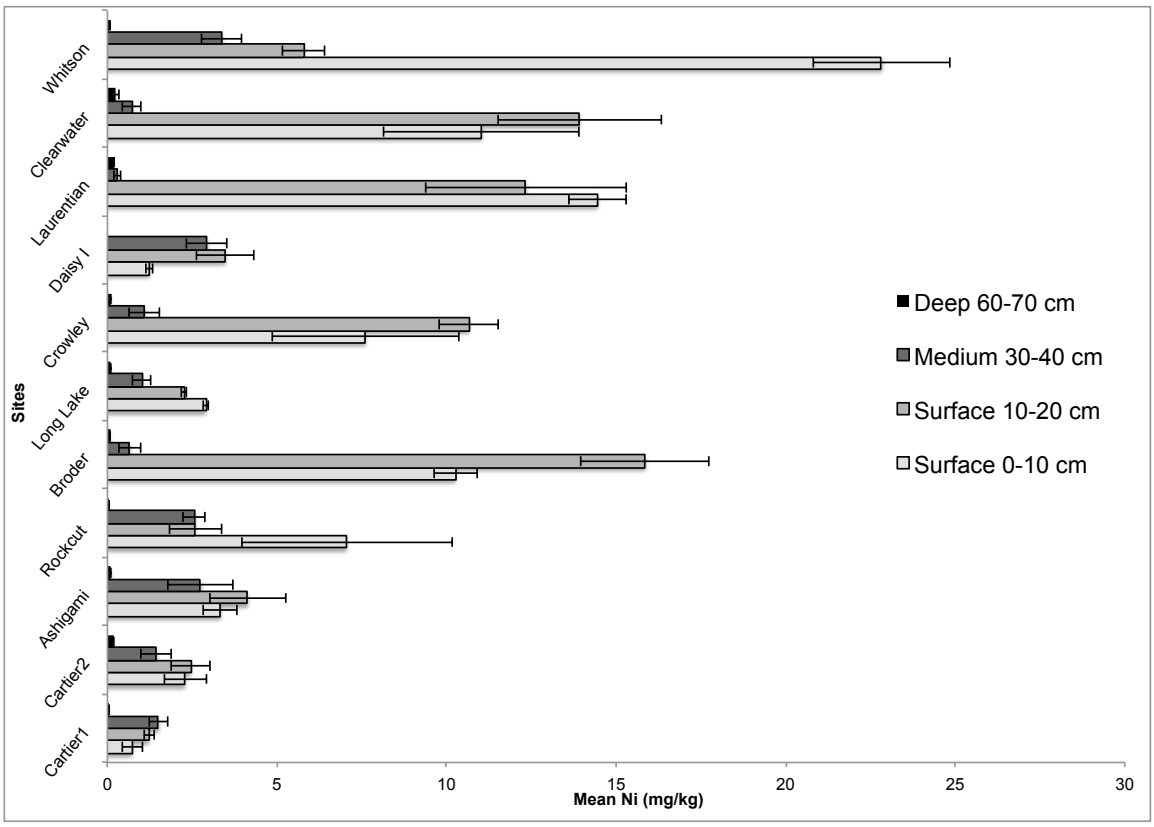



**Figure 3.7**- Peat Ni concentrations across eleven peatland sites at the four depths (surface 0-10 cm, subsurface 10-20 cm, medium 30-40 cm and deep 60-70 cm), ordered over a gradient of high-impacted (top of figure) to low-impacted (bottom of figure) sites. Error bars represent SE of the mean.

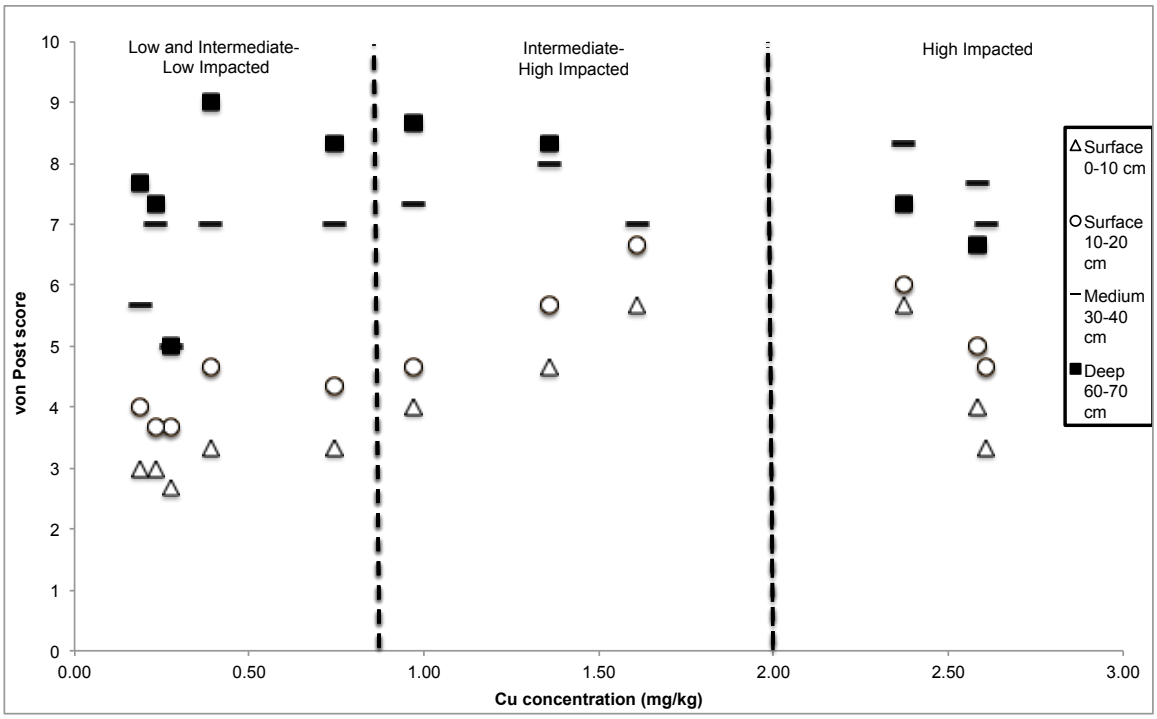



**Figure 3.8**-The relationship of von Post humification index with the four depth increments and surface Cu concentration at each of the eleven sites.

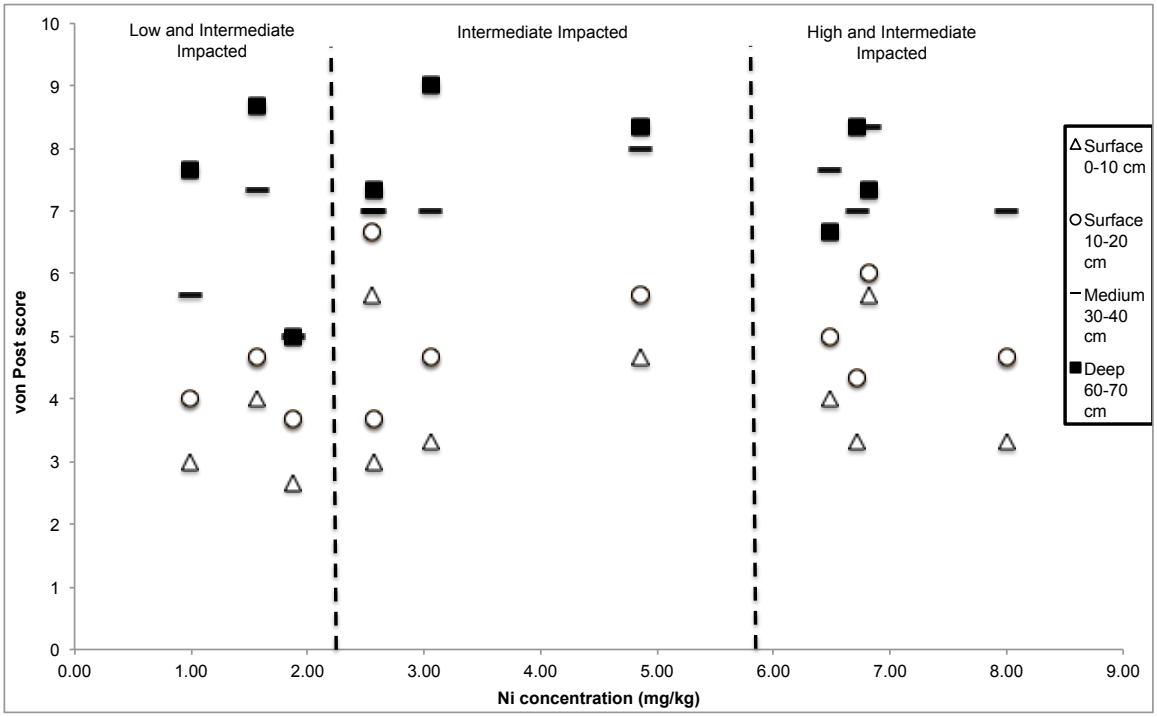



**Figure 3.9**-The relationship of von Post humification index with the four depth increments and surface Ni concentration at each of the eleven sites.

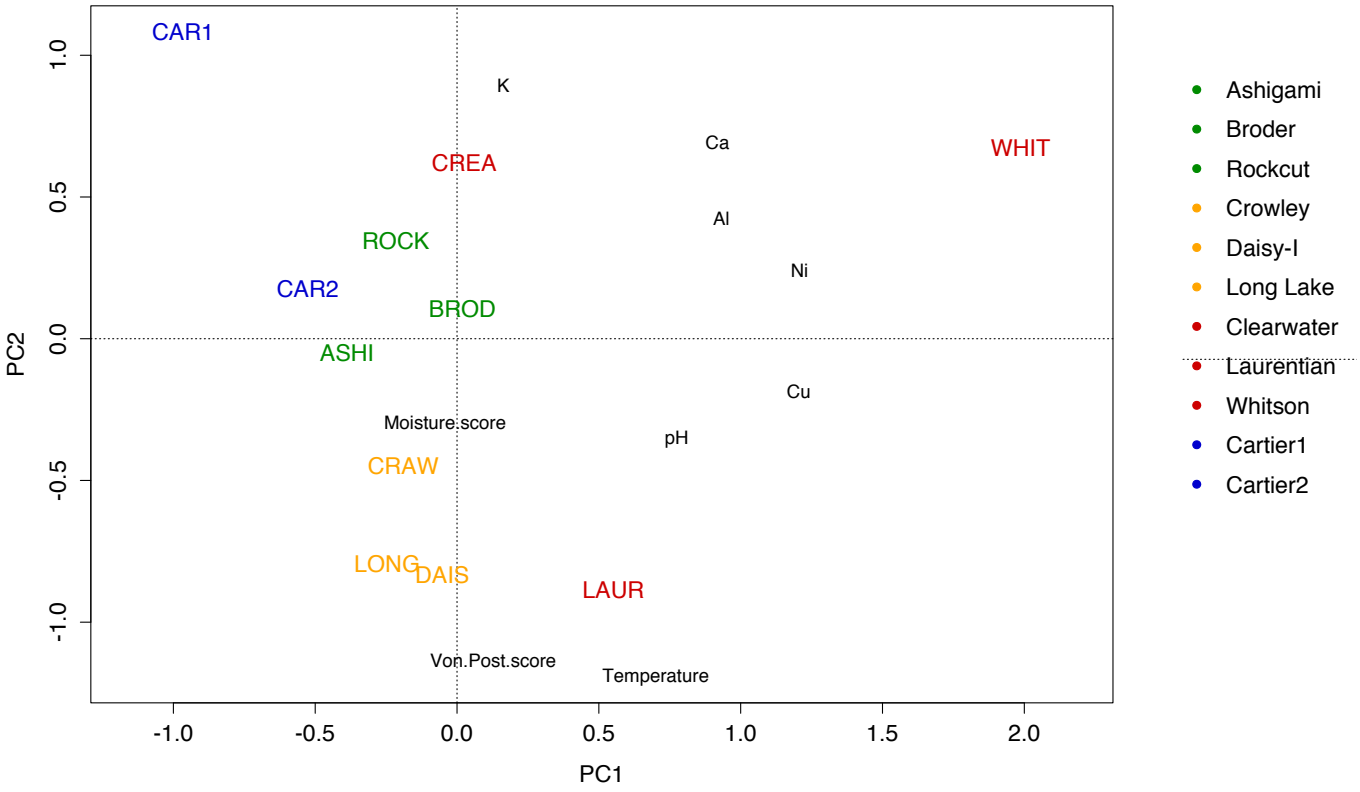



**Figure 3.10**- Principal component analysis for peat chemical and physical properties showing the variables and the peatland site loadings over a 0-10 cm depth. PC1 and PC2 explain 36.84 % and 22.33% proportion of variance respectively.

**Table 3.5**- PCA factor loadings for peat chemical and physical variables over a depth range from 0-10 cm. PC1 and PC2 explain 36.84 % and 22.33% proportion of variance of the variables respectively.

| **Variable** | **PC1** | **PC2** |
|---|---|---|
| Al | 0.39 | 0.20 |
| Cu | 0.50 | -0.09 |
| Ni | 0.50 | 0.11 |
| Ca | 0.38 | 0.33 |
| K | 0.67 | 0.42 |
| pH | 0.32 | -0.17 |
| Temperature | 0.29 | -0.56 |
| Von Post | 0.53 | -0.54 |
| Moisture | -0.02 | -0.14 |

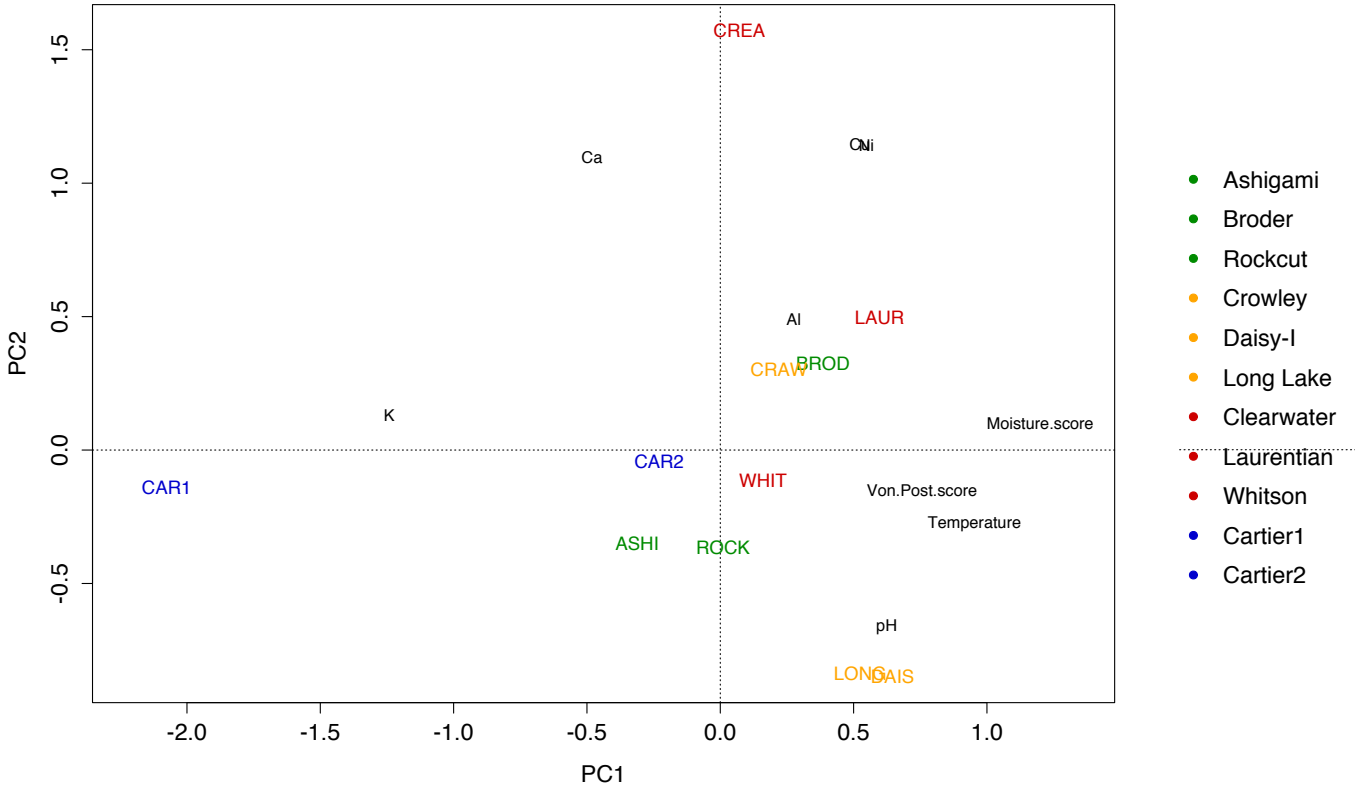



**Figure 3.11**- Principal component analysis for peat chemical and physical properties showing the variables and the peatland site loadings over a 10-20 cm depth. PC1 and PC2 explain 36.55% and 23.81% proportion of variance respectively.

**Table 3.6**- PCA factor loadings for peat chemical and physical variables over a depth range from 10-20 cm. PC1 and PC2 explain 36.55% and 23.81% proportion of variance of the variables respectively.

| **Variable** | **PC1** | **PC2** |
|---|---|---|
| Al | 0.12 | 0.23 |
| Cu | 0.22 | 0.53 |
| Ni | 0.23 | 0.53 |
| Ca | -0.20 | 0.51 |
| K | -0.52 | 0.06 |
| pH | 0.26 | -0.31 |
| Temperature | -0.40 | -0.13 |
| Von Post | 0.32 | -0.07 |
| Moisture | 0.50 | 0.05 |

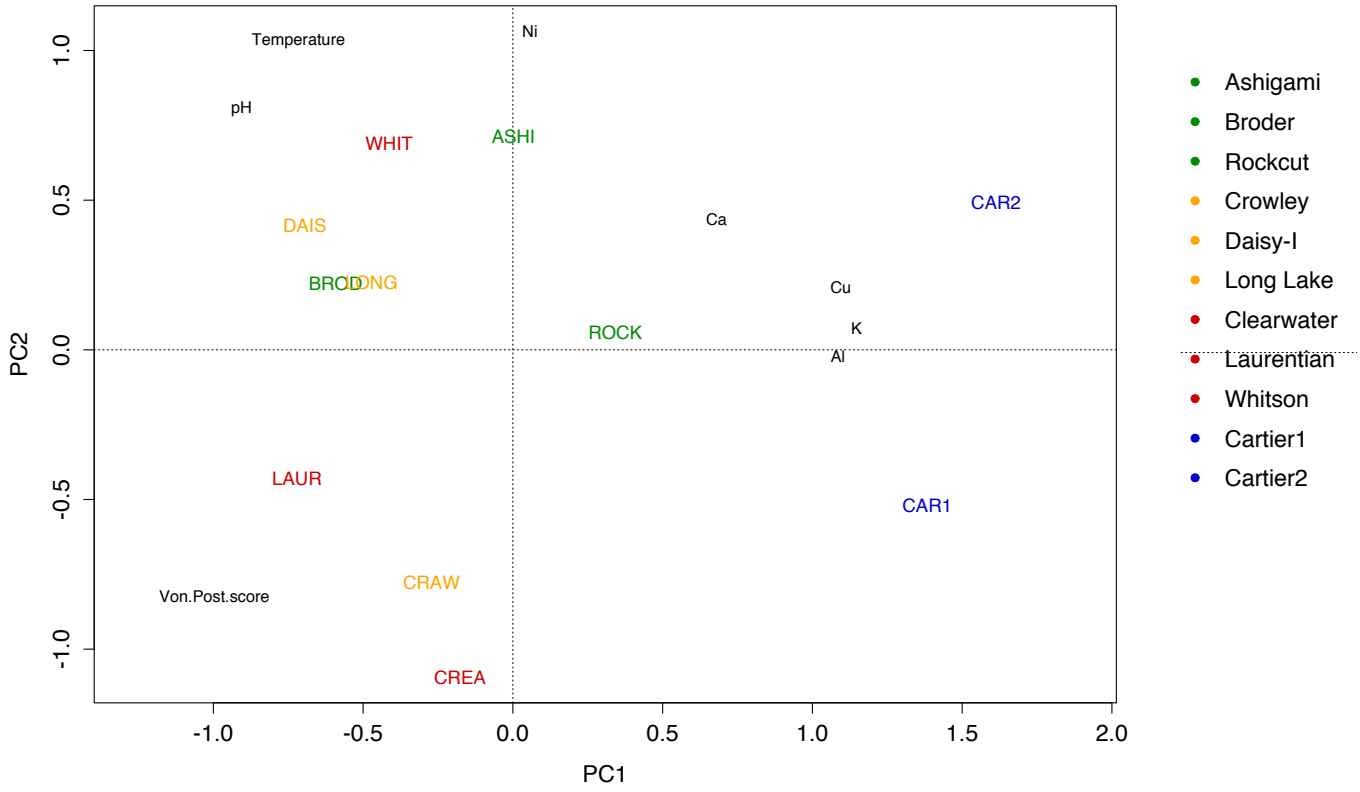



**Figure 3.12**- Principal component analysis for peat chemical and physical properties showing the variables and the peatland site loadings over a 30-40 cm depth. PC1 and PC2 explain 52.57% and 17.66% proportion of variance respectively.

**Table 3.7**- PCA factor loadings for peat chemical and physical variables over a depth range from 30-40 cm. PC1 and PC2 explain 52.57% and 17.66% proportion of variance of the variables respectively.

| **Variable** | **PC1** | **PC2** |
|---|---|---|
| Al | 0.43 | -0.01 |
| Cu | 0.43 | 0.11 |
| Ni | 0.02 | 0.55 |
| Ca | 0.27 | 0.23 |
| K | 0.45 | 0.04 |
| pH | -0.36 | 0.41 |
| Temperature | -0.28 | 0.53 |
| Von Post | -0.39 | -0.42 |
| Moisture | ---- | ---- |

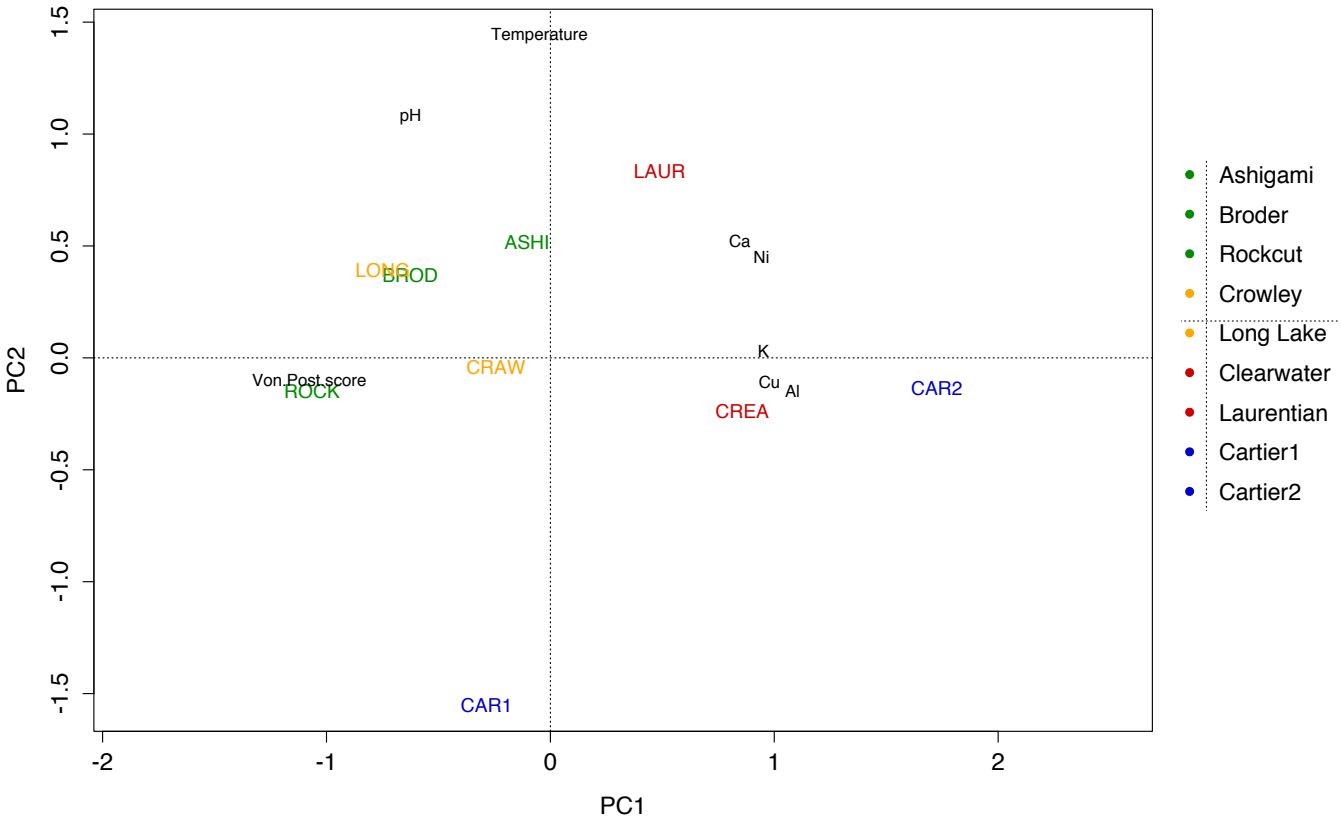



**Figure 3.13**- Principal component analysis for peat chemical and physical properties showing the variables and the peatland site loadings over a 60-70 cm depth (Daisy-I and Whitson not present at depth). PC1 and PC2 explain 60.26% and 22.12% proportion of variance respectively.

**Table 3.8**- PCA factor loadings for peat chemical and physical variables over a depth range from 60-70 cm. PC1 and PC2 explain 60.26% and 22.12% proportion of variance of the variables respectively.

| **Variable** | **PC1** | **PC2** |
|---|---|---|
| Al | 0.43 | -0.08 |
| Cu | 0.39 | -0.06 |
| Ni | 0.38 | 0.23 |
| Ca | 0.34 | 0.27 |
| K | 0.38 | 0.02 |
| pH | -0.25 | 057 |
| Temperature | -0.02 | 0.74 |
| Von Post | -0.43 | -0.05 |
| Moisture | ---- | ---- |

## 3.2 Microbial Community Structure and Diversity Analysis

### 3.2.1 Microbial Community Composition

The microbial dataset was filtered to remove unknown microbes at the kingdom and phylum levels, specifically, keeping 9166 classified OTUs from the 11 study sites. In order to analyze microbial community composition through ordinations, principal coordinate analysis (PCoA) was used to observe the effects of both the horizontal and vertical gradients over the study sites. There was a clear separation of the microbial communities between the low, both intermediate group sites, and high impact sites (Figure 3.12; Figure 3.14). However, the microbial communities at Clearwater (high), Crowley (intermediate- high) and Broder (intermediate- low) were observed to have similar microbial communities across sites, which could be due to similarities in chemical and physical properties in general (Figure 3.12). Additionally, within these groups, a vertical gradient was also apparent with clear patterns within the microbial communities identified in the shallow depths compared to the intermediate and deep peat samples (Figure 3.13). This was consistent across all of the sites.

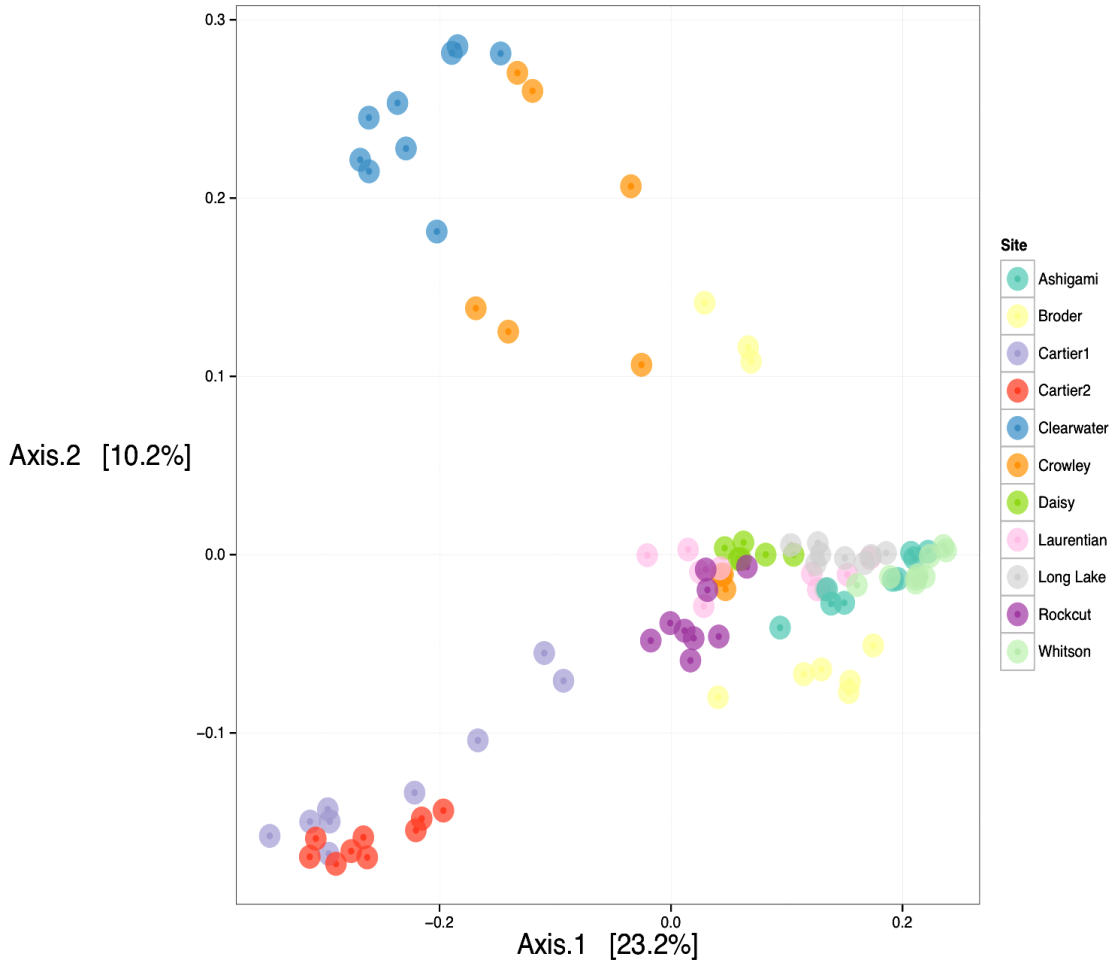



**Figure 3.14**- The relationships between microbial (bacterial and archaeal) communities associated with 11 peatland sites in the Sudbury area. The communities represent the full data after quality filtering of singletons and chimeras. The PCoA of the unweighted UniFrac distance matrix was used to cluster the microbial communities across the different sites.

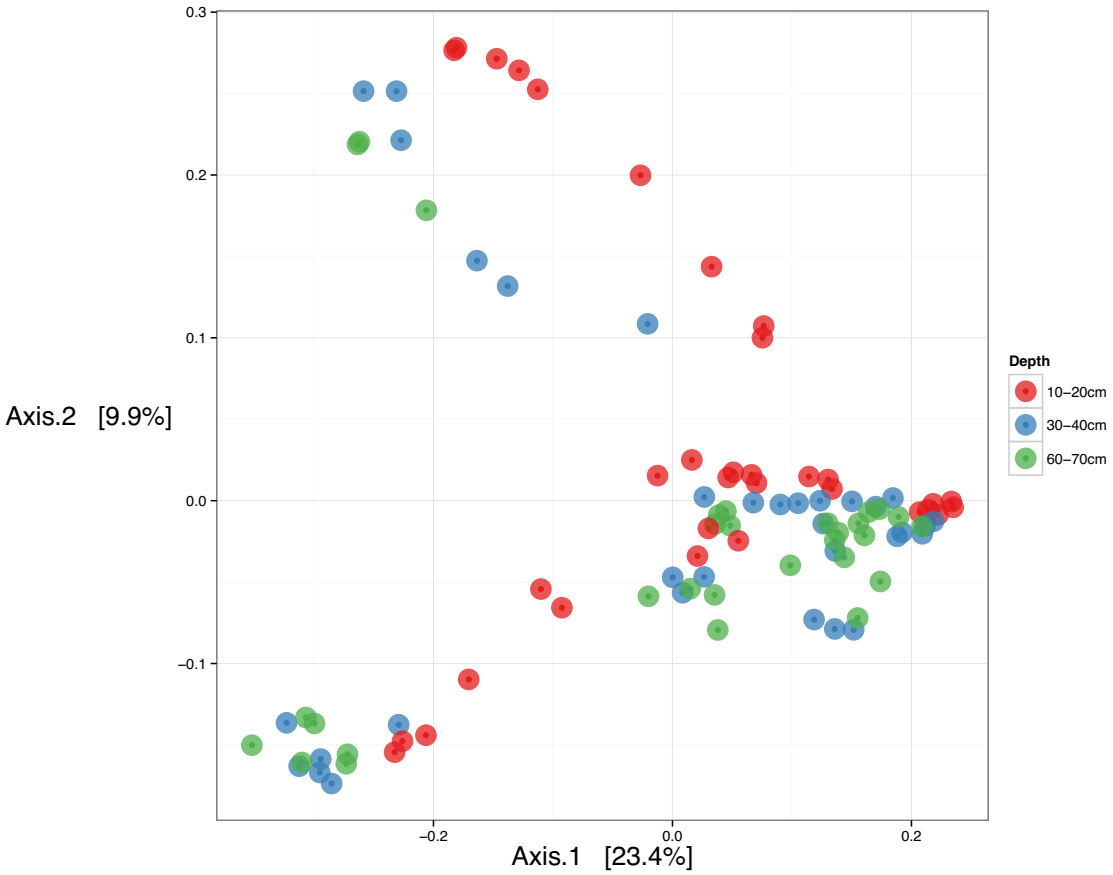



**Figure 3.15**- The relationship between microbial (bacterial and archaeal) communities associated with 3 depths over different peatlands in the Sudbury area. The communities represent the full data after quality filtering of singletons and chimeras. The PCoA of the unweighted UniFrac distance matrix was used to cluster the microbial communities over the different depths.

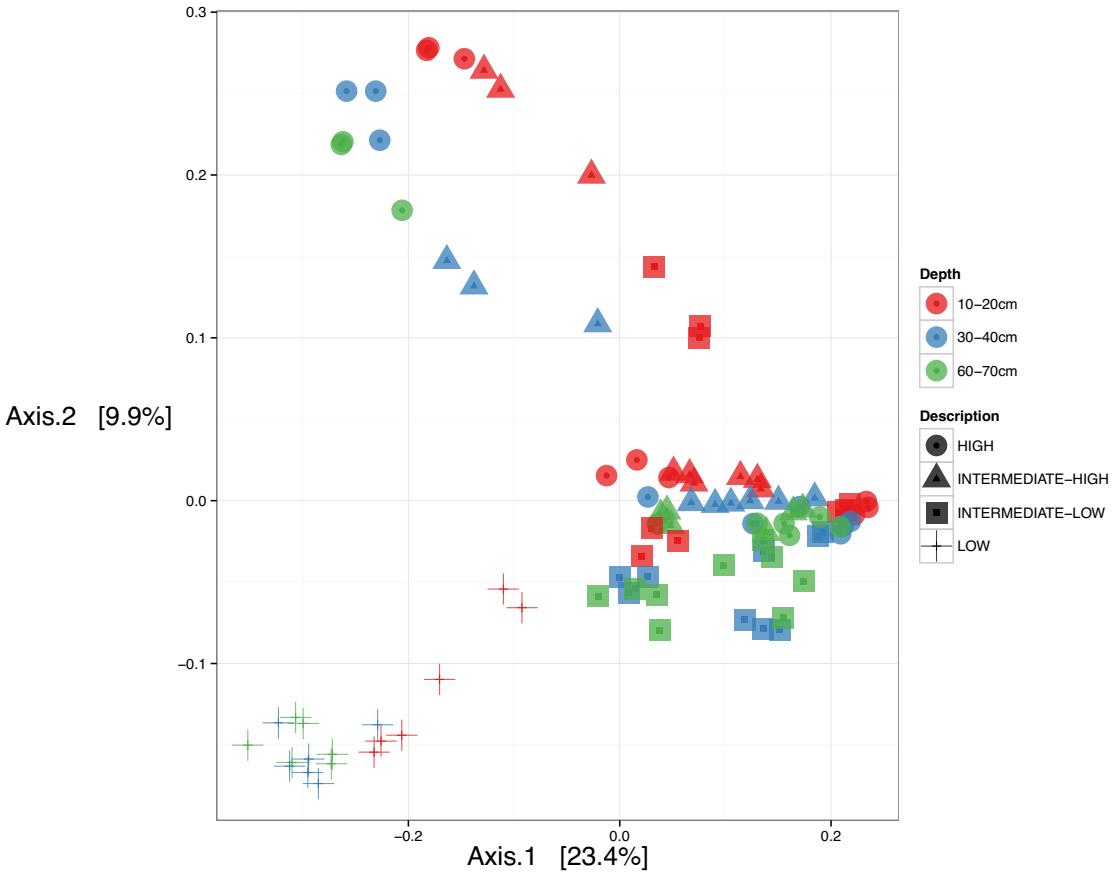



**Figure 3.16**- The interaction between microbial (bacterial and archaeal) communities associated with 3 peatland depths across 11 sites over unique groupings ranging from high, intermediate- high, intermediate-low and low-impacted in the Sudbury area. The communities represent the full data after quality filtering of singletons and chimeras. The PCoA of the unweighted UniFrac distance matrix was used to cluster the microbial communities over the different depths, sites and descriptions.

### 3.2.2 Microbial Community Diversity

Shannon diversity index was used to observe the diversity of the microbial communities across the metal gradient from high-impacted to low-impacted sites for 9166 OTUs. A curved trend was observed with a higher mean diversity in the intermediate-high and intermediate-low sites (Figure 3.15). The high-impacted sites (closest to the Copper Cliff smelter) had generally lower microbial diversity when compared to the other sites, except for Whitson, which had the highest microbial diversity of all 11 sites. The two low-impacted sites (furthest from the Copper Cliff smelter) were observed to have the lowest Shannon diversity indices of all 11 sites studied (Figure 3.15).

Shannon diversity index was also used to observe the diversity of the microbial community for the 9166 OTUs across the three depth increments at each site. There was no difference observed for the mean diversity of microbes across the three depths from surface to deep peat (10-20 cm, 30-40 cm and 60-70 cm), but there was an increase in variation from the mean with depth (Figure 3.16).

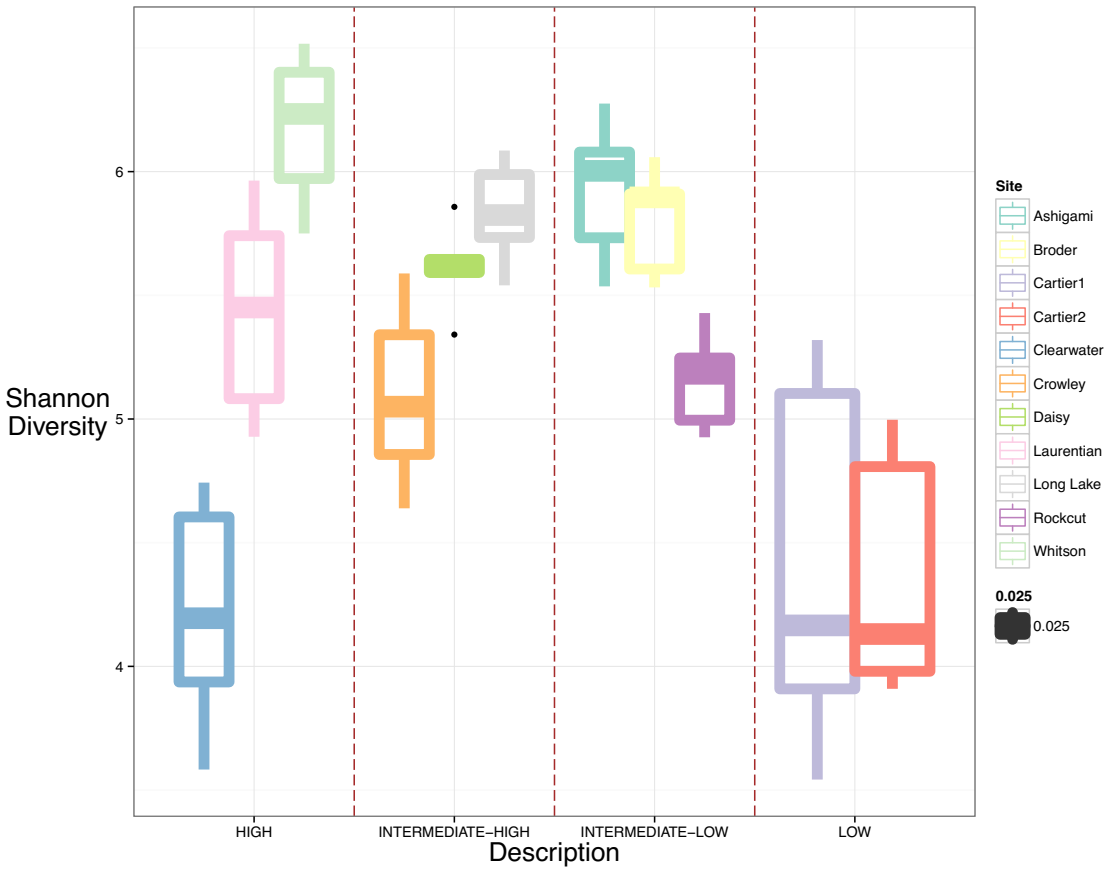



**Figure 3.17**- Shannon diversity index showing microbial diversity over 11 peatland sites in the Sudbury area, ranging from high (right-side of the figure) to low (left-side of the figure) impacted sites. The microbial diversity represents the diversity of the full data after quality filtering of singletons and chimeras.

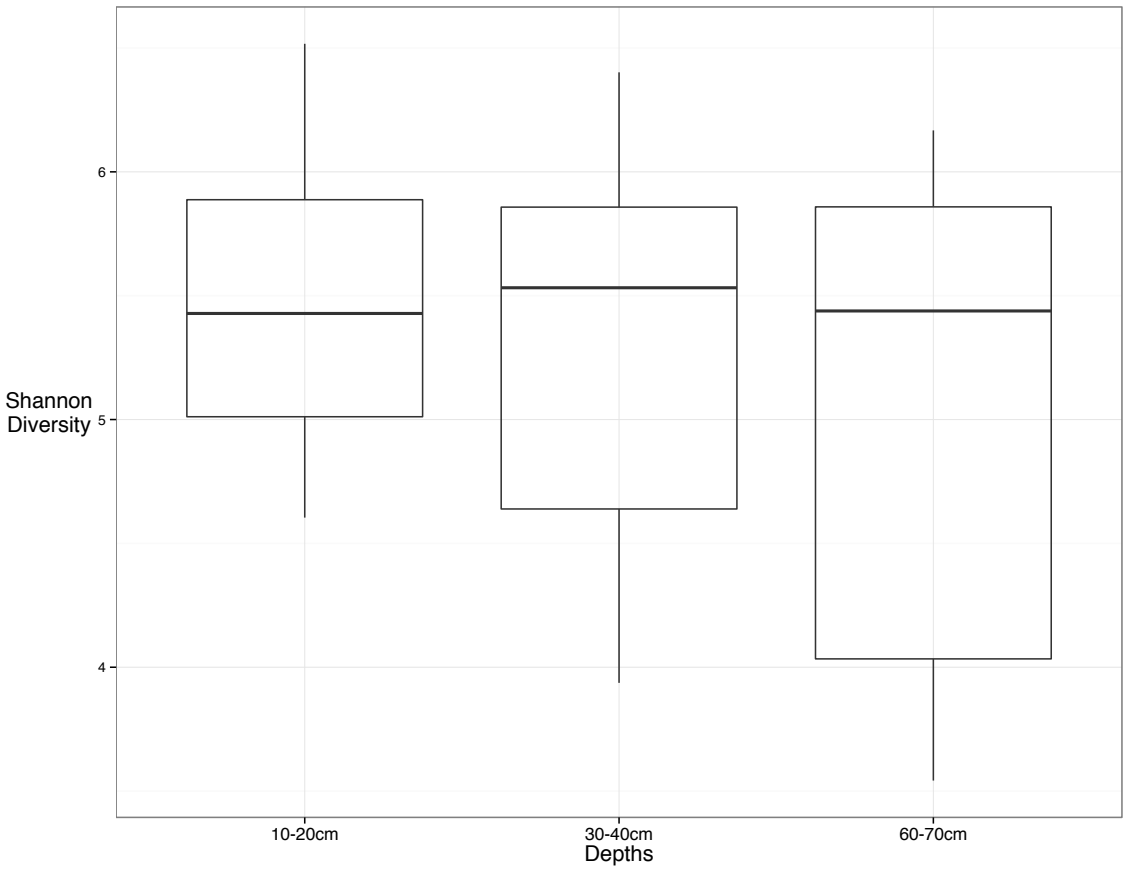



**Figure 3.18**- Shannon diversity index showing microbial diversity over three depths in the Sudbury area, ranging from surface (10-20 cm), subsurface (30-40 cm) to deep (60-70 cm). The microbial diversity represents the diversity of the full data after quality filtering of singletons and chimeras.

## 3.3 Microbial Phylogeny and its Distribution

### 3.3.1 Phylogenetic Abundances and Distribution of Microbial Species

The microbial dataset was filtered to remove low abundance microbes, retaining OTUs with a relative percent abundance of > 1% within each of the 11 study sites, giving a total of 45 OTUs. These 45 OTUs represented 40.1% of the total abundance for the original 9166 OTU data set.

The low-impacted sites had high abundance of Acidobacteria and Proteobacteria; with Cartier1 also having abundant Actinobacteria, Nitrospirae, and Planctomycetes at the phylum level (Figure 3.17). Cartier1 also had an abundance of Crenarchaeota and Euryarchaeota at depth (Figure 3.17). This trend of abundant taxa was seen throughout all taxonomic levels including at the order level with organisms such as Syntrophobacterales and Rhizobiales from the phylum Proteobacteria, Ellin6513 and Acidobacteriales from the phylum Acidobacteria, MSBL9 (phylum Planctomycetes), Nitrospirales (phylum Nitrospirae), Solirubrobacterales (phylum Actinobacteria) and NRP-J (phylum Crenarchaeota) at depth (Figure 3.19). There was an abundance of NC10, Verrucomicrobia, Euryarchaeota and Crenarchaeota (at depth) in Cartier2 (Figure 3.17). Additionally, this trend was seen throughout all taxonomic levels with an abundance of Methylocystaceae, Hyphomicrobiaceae, Syntrophobacteraceae, Syntrophaceae and Bradyrhizobiaceae (phylum Proteobacteria) at the family level (Figure 3.20). Cartier2 was also abundant in Acidobacteriaceae from the phylum Acidobacteria, Ellin515 (phylum Verrucomicrobia), and Methanomassiliicoccaceae (phylum Euryarchaeota) (Figure 3.20).

At the phylum level, the intermediate-low impacted sites showed an abundance of Acidobacteria and Proteobacteria (Figure 3.17). At the class level, organisms such as Alphaproteobacteria (phylum Proteobacteria) and Acidobacteriia and Solibacteres (phylum Acidobacteria) were fairly abundant across the sites (Figure 3.18). There was a unique abundance of Dehalococcoidetes (phylum Chloroflexi), MBGA (phylum Crenarchaeota),

Nitrospira (phylum Nitrospirae), DA052 (phylum Acidobacteria) and Thermoplasmata (phylum Euryarchaeota) in Ashigami (Figure 3.18). There was also a unique abundance of Betaproteobacteria and Deltaproteobacteria (phylum Proteobacteria), TM1 (phylum Acidobacteria), MBGA (phylum Crenarchaeota) and 12-24 (phylum NC10) in Rockcut; while Broder had an abundance of 12-24 (phylum NC10), Deltaproteobacteria (phylum Proteobacteria), and Nitrospira (phylum Nitrospirae) (Figure 3.18). Additionally, these trends continued to the genus level, where there was an abundance of organisms from the phylum Proteobacteria, including *Bradyrhizobium*, *Geobacter*, *Desulfobacca* and *Syntrophobacter* in Rockcut, *Candidatus Koribacter* and *Candidatus Solibacter* (phylum Acidobacteria) were abundant in Ashigami, and *Rhodoplanes* was abundant in Ashigami and Broder (Figure 3.21).

In the intermediate-high impacted sites, there was an abundance of Acidobacteria, Crenarchaeota and Proteobacteria at the phylum level across all sites, and an abundance of Euryarchaeota, AD3 and Nitrospirae in Crowley, Chlorobi in Long Lake, and Actinobacteria, Chloroflexi, Crenarchaeota and Euryarchaeota in Daisy-I (Figure 3.17). This trend was observed throughout all taxonomic levels, including the family level, with organisms such as Syntrophobacteraceae, Bradyrhizobiaceae and Hyphomicrobiaceae, and Syntrophaceae (phylum Proteobacteria), Koribacteraceae and AKIW659 (phylum Acidobacteria) and Methanomassiliicoccaceae (phylum Euryarchaeota) being abundant in Crowley (Figure 3.20). Organisms such as Acidobacteriaceae and Koribacteraceae (phylum Acidobacteria), and Bradyrhizobiaceae, Hyphomicrobiaceae and Sinobacteraceae (phylum Proteobacteria) were abundant in Long Lake, while Methanomassiliicoccaceae (phylum Euryarchaeota), Syntrophobacteraceae and Syntrophaceae (phylum Proteobacteria) and Dehalococcoidaceae (phylum Chloroflexi) were abundant in Daisy-I (Figure 3.20).

There was an abundance of Acidobacteria and Proteobacteria in the high-impacted sites at the phylum level; and also an abundance of Actinobacteria, Nitrospirae, Chlorobi and Crenarchaeota at Laurentian, Euryarchaeota, Crenarchaeota and Bacteroidetes at Whitson, and

Euryarchaeota and Crenarchaeota at Clearwater (Figure 3.17). These trends were observed across all taxonomic levels in the high-impacted sites, for example at the family level with Koribacteraceae (phylum Acidobacteria), Gaiellaceae (phylum Actinobacteria), Hyphomicrobiaceae, Syntrophobacteraceae, Methylocystaceae (phylum Proteobacteria), and Thermodesulfovibrionaceae (phylum Nitrospirae) were observed at Laurentian (Figure 3.20). Organisms such as Hyphomicrobiaceae, Methylocystaceae, Syntrophaceae and Syntrophobacteraceae (phylum Proteobacteria), Koribacteraceae, AKIW659 and Solibacteraceae (phylum Acidobacteria), and Methanomassiliicoccaceae (phylum Euryarchaeota) were abundant in Clearwater, while organisms such as AKIW659 (phylum Acidobacteria), Hyphomicrobiaceae and Syntrophaceae (phylum Proteobacteria), and Methanomassiliicoccaceae (phylum Euryarchaeota) were abundant in Whitson (Figure 3.20).

Microbial taxa differed over the three depths studied (10-20 cm, 30-40 cm and 60-70 cm) ranging from an abundance of 0 – 0.1. The surface had an abundant (≤ 0.02) of E2, NRP-J, Ellin6513, Syntrophobacterales, Rhizobiales and Acidobacteriales across all the sites (Figure 3.22). Additionally, MSBL9, Solirubrobacterales, JH-WHS47, Pedosphaerales, Desulfuromonadales were abundant in the low and intermediate-low impacted sites, Xanthomonadales was abundant in the low-impacted sites, and Dehalococcoidales and Acidimicrobiales were abundant in the high-impacted sites (Figure 3.22).

In the medium depth (30-40 cm), Nitrospirales, Syntrophobacterales, Rhizobiales, Acidobacteriales, Ellin6513, E2 and NRP-J were abundant (≤ 0.02) across all the sites (Figure 3.23). MSBL9, Solirubrobacterales, JH-WHS47 and Pedosphaerales were abundant in the intermediate- low and low-impacted sites, while Gaiellales was abundant in the intermediate-high impacted sites. Lastly, Bacteroidales was abundant in the high-impacted sites (Figure 3.23).

In the deep peat depth (60-70 cm), Sbla14, Nitrospirales, Syntrophobacterales, Rhizobiales, Acidobacteriales, Ellin6513, E2, Solibacterales and NRP-J were abundant (≤ 0.02) across all the sites (Figure 3.24). Pedosphaerales was abundant in the low-impacted sites, and

JH-WHS47 was abundant in the intermediate- low and low-impacted sites. Additionally, Dehalococcoidales was abundant in the intermediate-low impacted sites, while Xanthomonadales was abundant in the intermediate-high impacted sites, and Bacteroidales and Solirubrobacterales were abundant in the high-impacted sites (Figure 3.24).

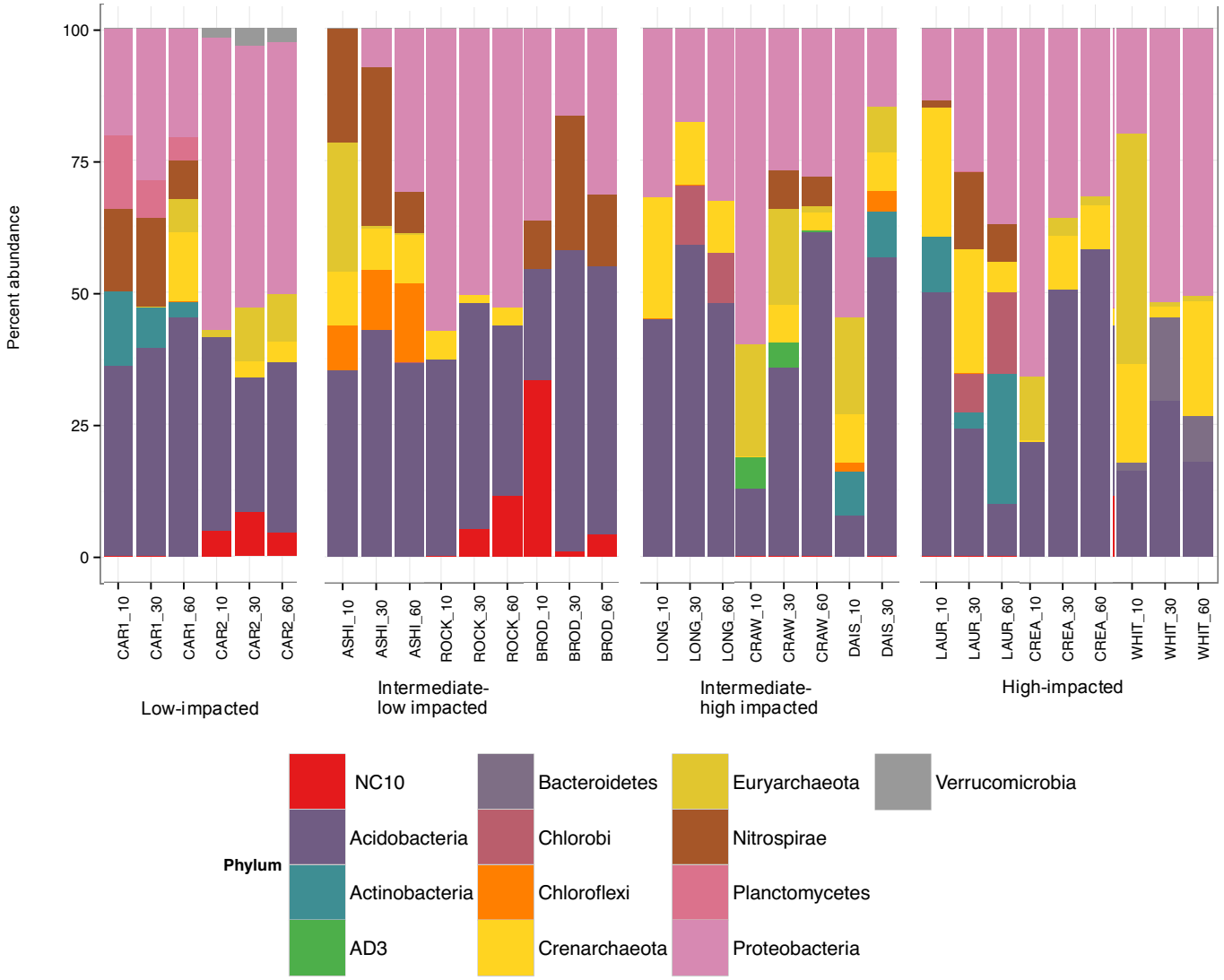



**Figure 3.19-** Percent relative abundance of the taxonomic composition at the phyla level for microbial communities associated with 11 peatland sites in the Sudbury area, ranging from a gradient of metal from low (left-side of figure) to high (right-side of figure) impacted sites.

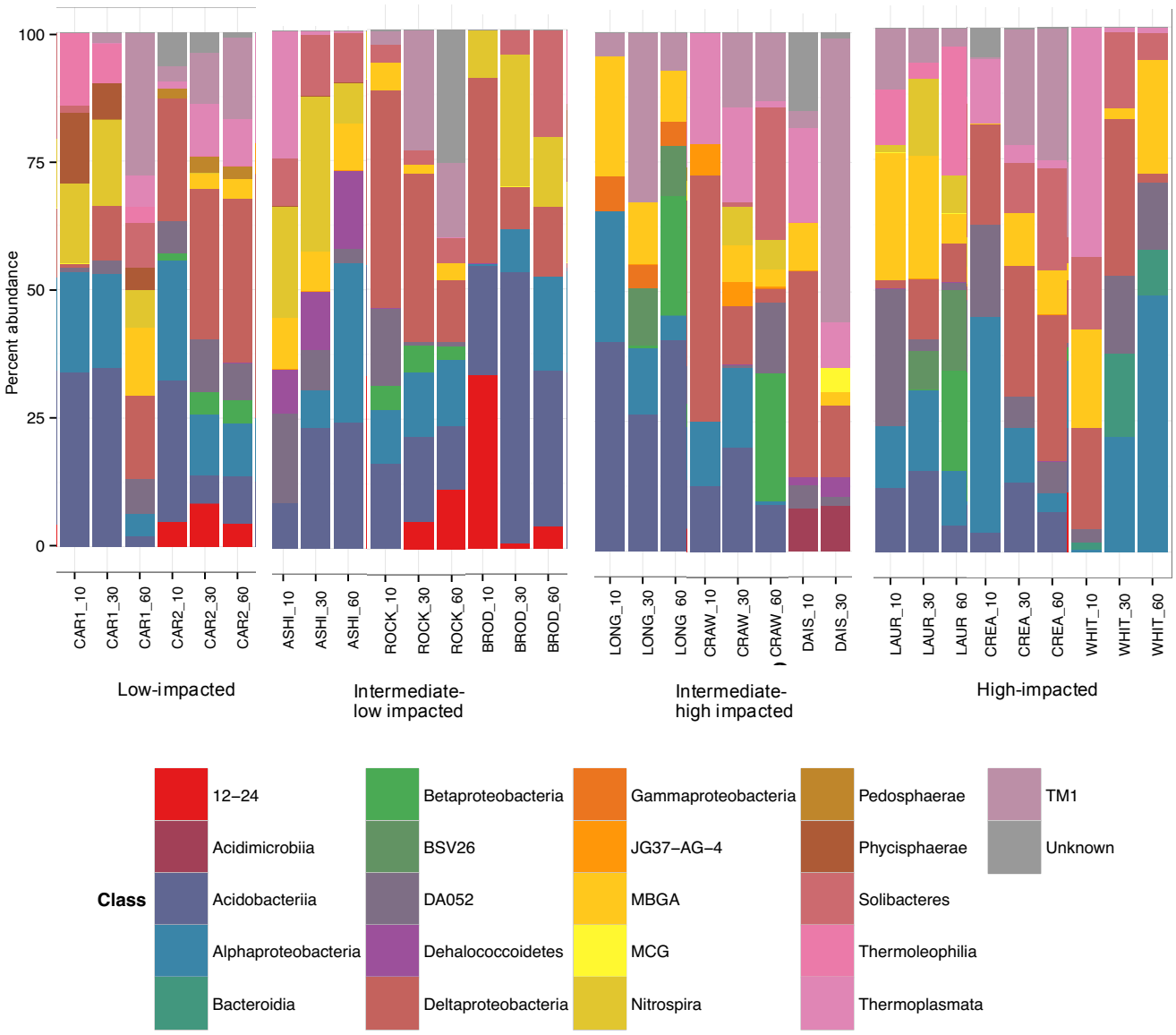



**Figure 3.20**- Percent relative abundance of the taxonomic composition at the class level for microbial communities associated with 11 peatland sites in the Sudbury area, ranging from a gradient of metal from low (left-side of figure) to high (right-side of figure) impacted sites.

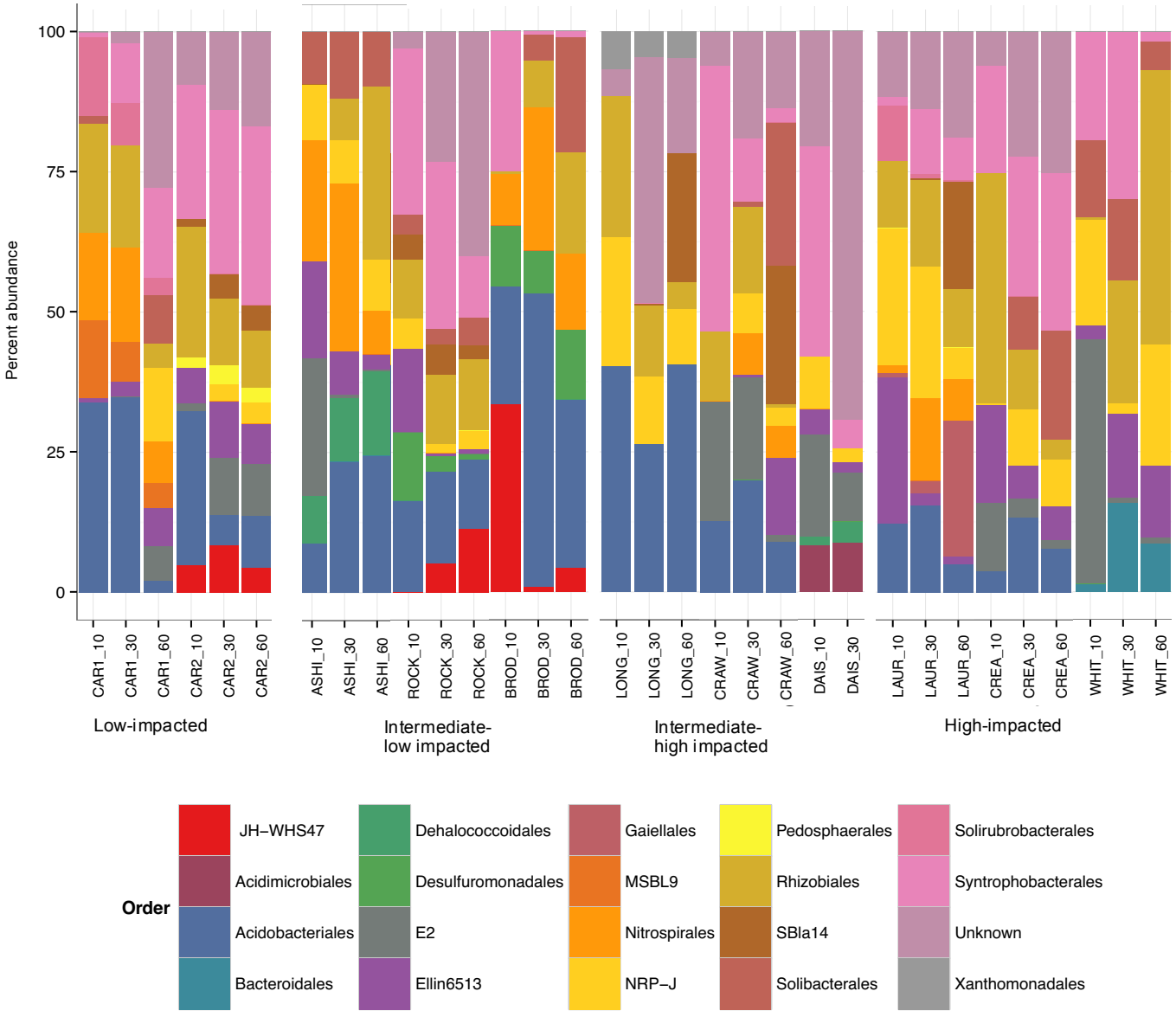



**Figure 3.21**- Percent relative abundance of the taxonomic composition at the order level for microbial communities associated with 11 peatland sites in the Sudbury area, ranging from a gradient of metal from low (left-side of figure) to high (right-side of figure) impacted sites.

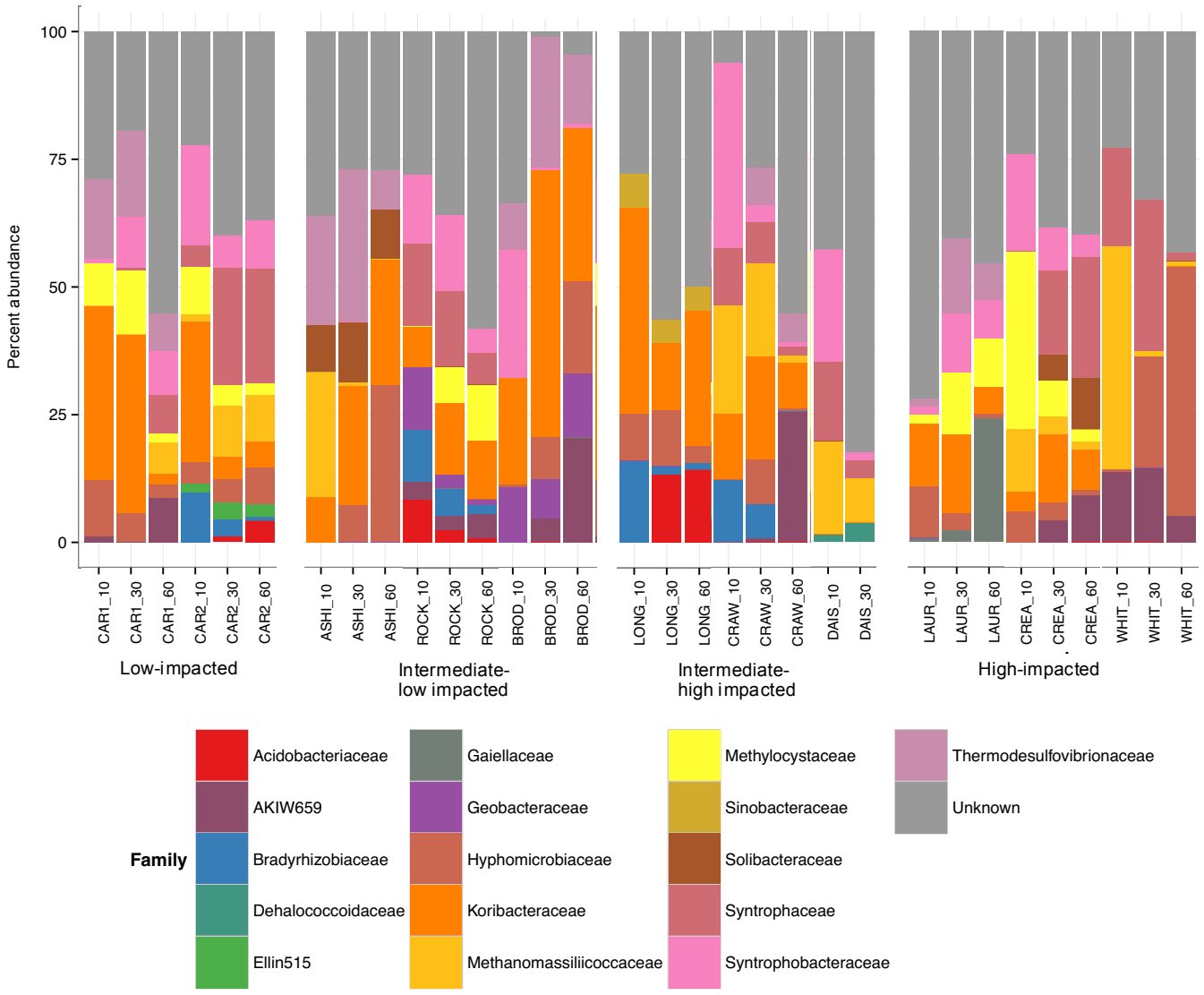



**Figure 3.22**- Percent relative abundance of the taxonomic composition at the family level for microbial communities associated with 11 peatland sites in the Sudbury area, ranging from a gradient of metal from low (left-side of figure) to high (right-side of figure) impacted sites.

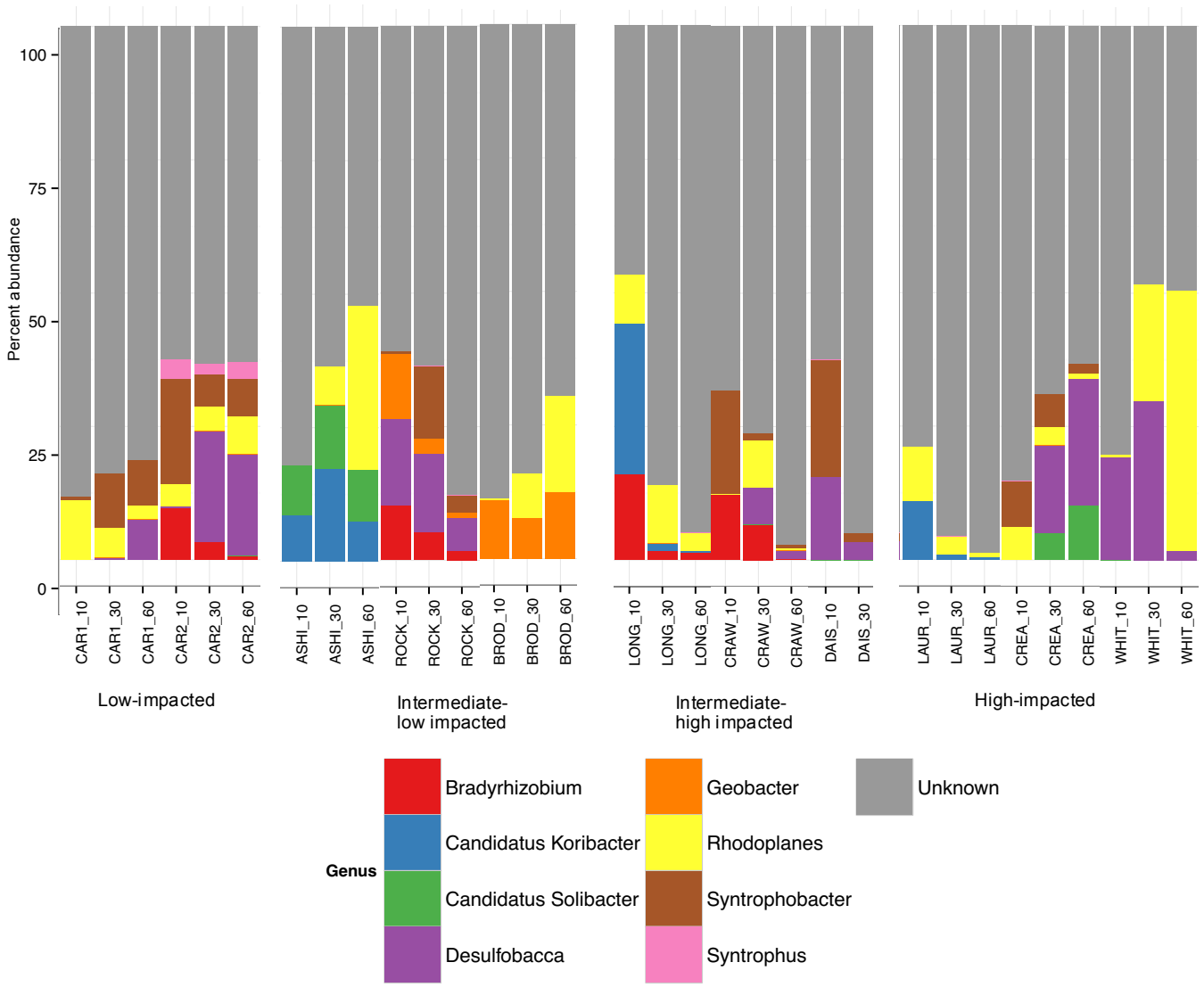



**Figure 3.23**- Percent relative abundance of the taxonomic composition at the genus level for microbial communities associated with 11 peatland sites in the Sudbury area, ranging from a gradient of metal from low (left-side of figure) to high (right-side of figure) impacted sites.

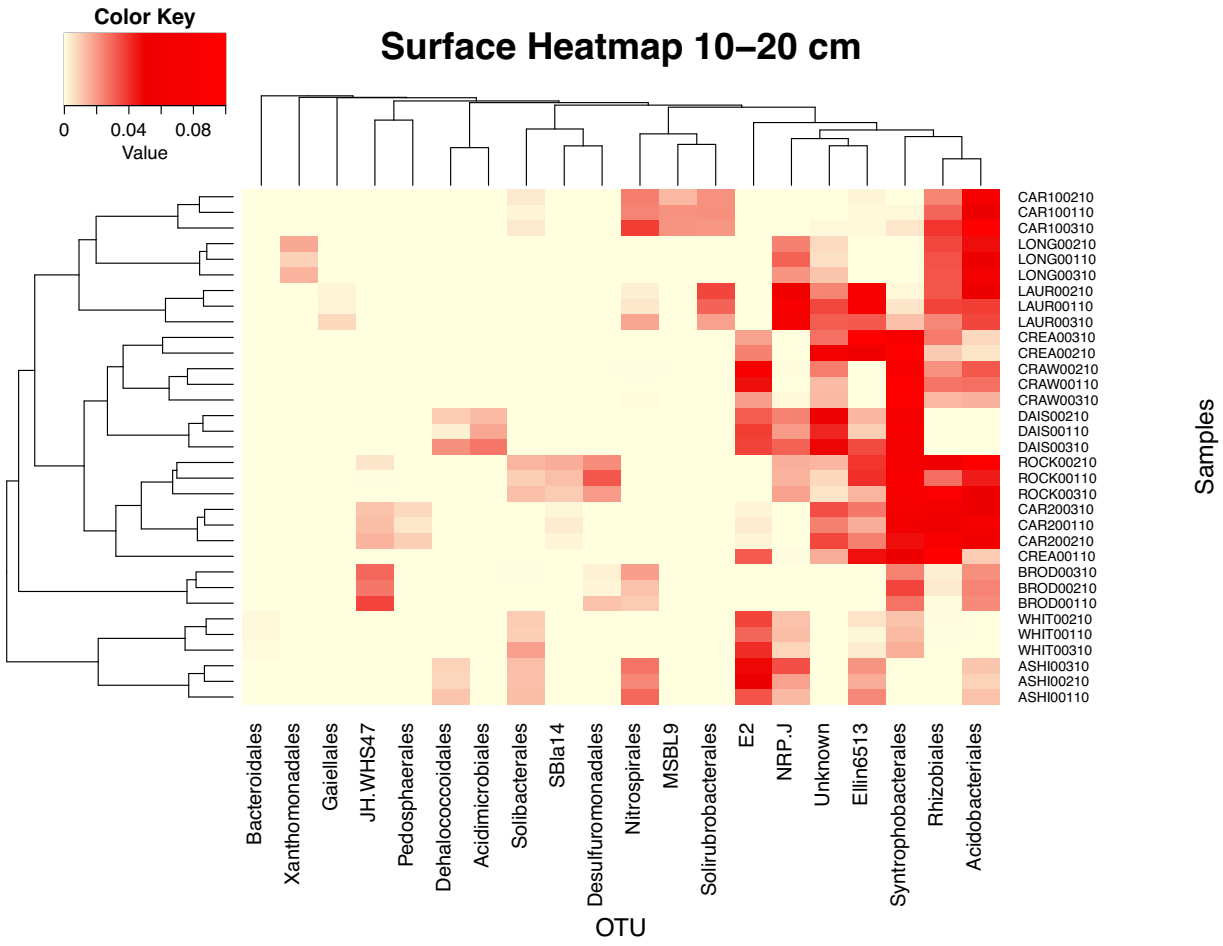



**Figure 3.24-** Heatmap of abundant OTUs (columns) across the 11 study sites with three replicates (rows) at the 10-20 cm depth increment, sorted by hierarchal clustering using Bray-Curtis. GreenGenes was used to assign taxonomy at the order level, and abundances were shown in the key as increasing from light (0.0) to dark (1.0).

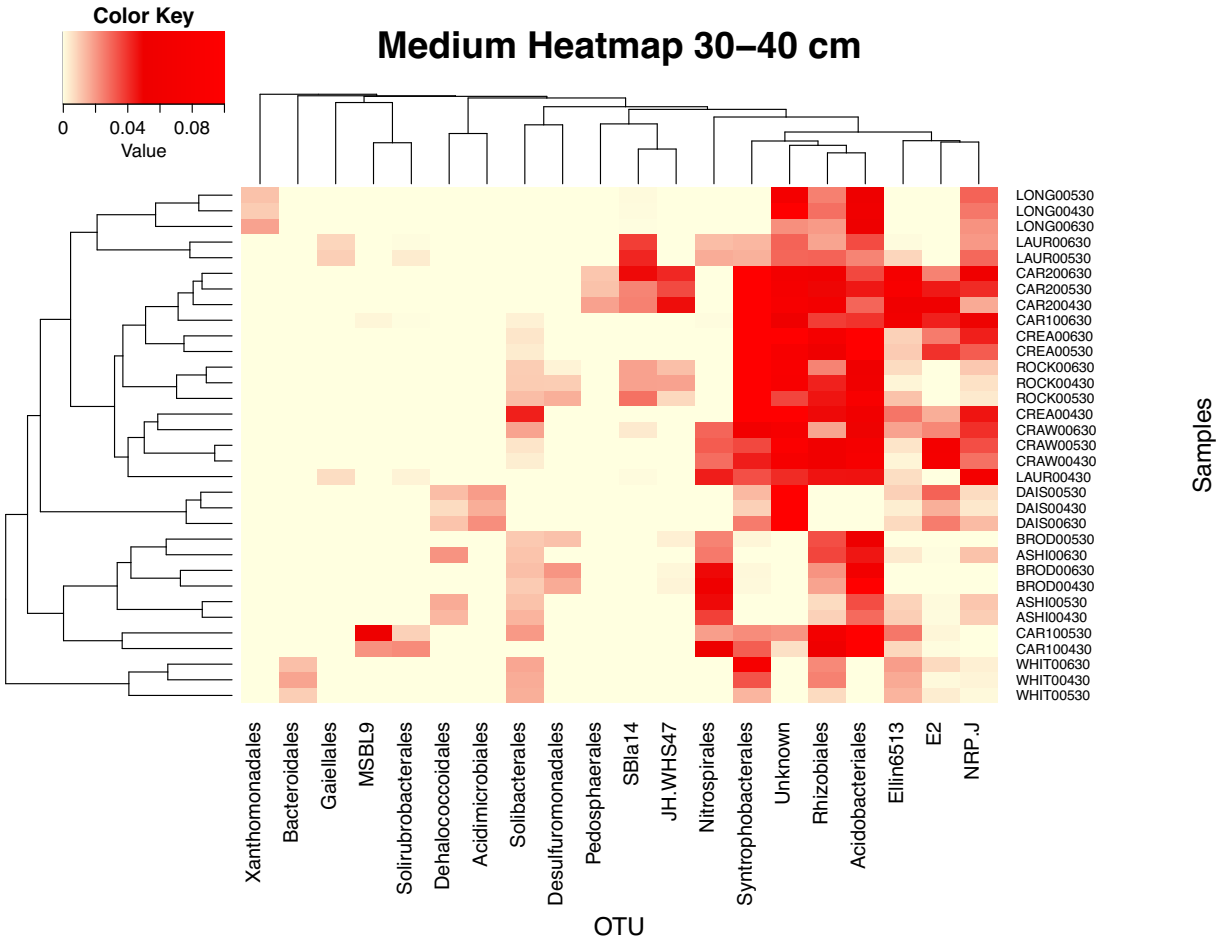



**Figure 3.25-** Heatmap of abundant OTUs (columns) across the 11 study sites with three replicates (rows) at the 30-40 cm depth increment, sorted by hierarchal clustering using Bray-Curtis. GreenGenes was used to assign taxonomy at the order level, and abundances were shown in the key as increasing from light (0.0) to dark (1.0).

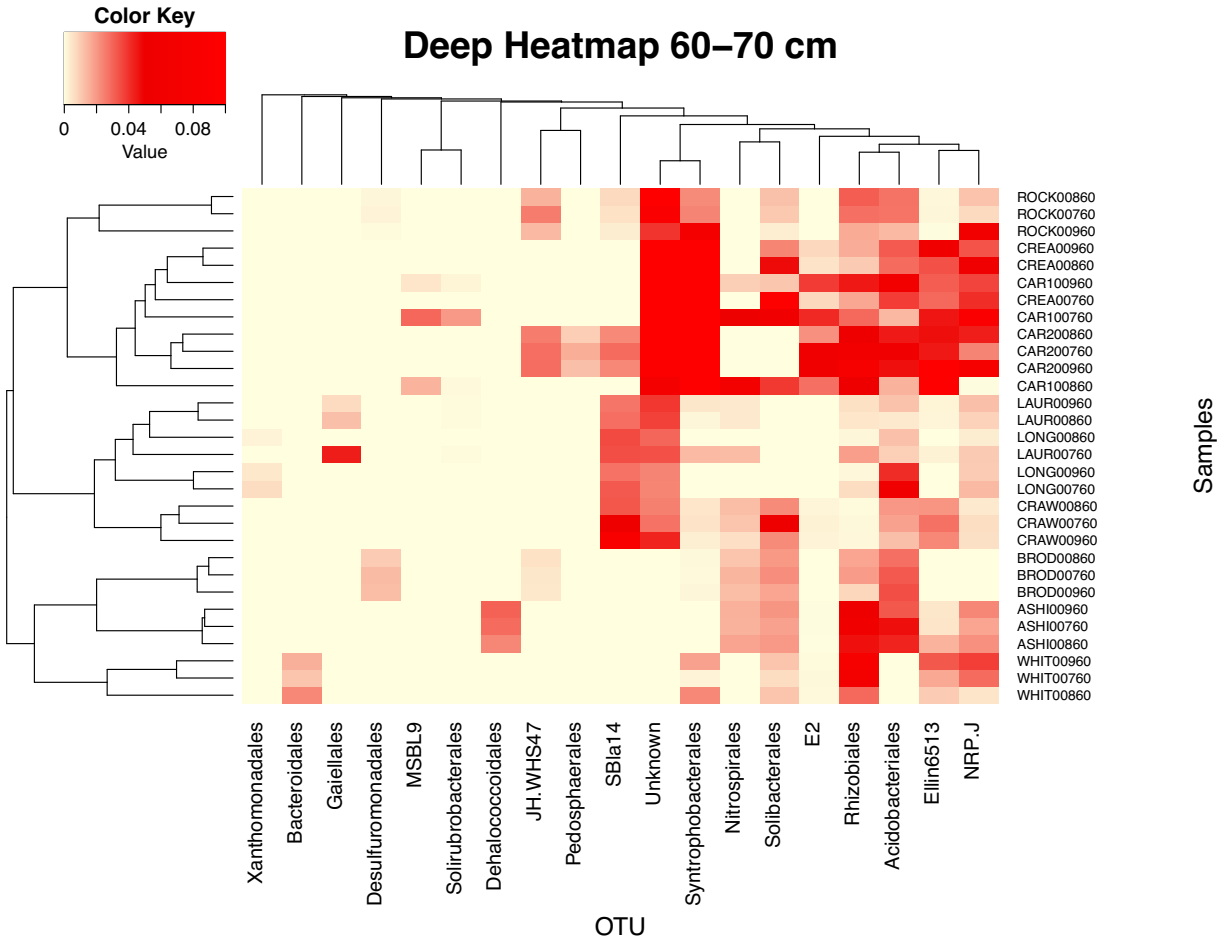



**Figure 3.26-** Heatmap of abundant OTUs (columns) across 10 study sites with three replicates (rows) at the 60-70 cm depth increment, sorted by hierarchal clustering using Bray-Curtis. The 11$^{th}$ study site (Daisy I) was excluded from this analysis because it was too shallow and samples could not be obtained. GreenGenes was used to assign taxonomy at the order level, and abundances were shown in the key as increasing from light (0.0) to dark (1.0).

### 3.3.2 Key Microbial Taxa and Their Presence Along a Metal Gradient

Ten potential bacterial indicators were observed across the different sites, depths and metal gradient ($p$-value < 0.05)**,** with the majority of the potential indicators existing across sites (Table 3.9). Five of the ten potential indicator species were associated with the study sites, and of these five, three belonged to the phylum Proteobacteria, and the remaining two belong to the phyla Acidobacteria and Verrucomicrobia (Table 3.9). Additionally, of the ten potential indicator species, two species were associated with depth, with one species (OTU 7) overlapping with the metal gradient. The potential indicator species associated with depth belonged to the phyla Acidobacteria and Proteobacteria (Table 3.9). Lastly, three of the ten potential indicator species were associated with the metal gradient present across the sites, with two of the three species belonging to phylum Acidobacteria and the third species belonging to phylum Planctomycetes (Table 3.9).

Phylogenetic analysis of the 45 nucleotide sequences in the > 1% OTUs and an additional 45 closely related (>85% identical) nucleotide sequences from GenBank (Appendix 7.7: Table 7.13 and Table 7.14) were studied using the 257 rRNA genes present in each sequence. The organisms belonging to the phylum Proteobacteria were monophyletic, with taxa such as Syntrophobacteraceae, Syntrophaceae, *Syntrophus*, *Syntrophobacter*, *Geobacter* and Deltaproteobacteria being closely related (Figure 3.25). Acidobacteria was polyphyletic, with organisms such as OTU7 and OTU21 (found in Rockcut), OTU8 and OTU74 (found in Crowley), OTU6 and OTU49 (found in Clearwater) sharing common hierarchy. Some Acidobacteria shared similar hierarchy but were found in different sites, for example, OTU34 (found in Laurentian and Long Lake) and OTU40 (found in Ashigami), OTU42 (found in Cartier2 and Rockcut) and OTU98 (found in Clearwater and Whitson). Additionally, Actinobacteria were distinct in the sites they belonged to, for example, OTU27 (found in Laurentian), OTU28 (found in Cartier1 and Laurentian) and OTU36 (found in Daisy-I). Similarly Chloroflexi (OTU25 found in Ashigami

and OTU86 found in Daisy-I) and Crenarchaeota (OTU59 found in Daisy-I, OTU50 found in Laurentian) had organisms that belonged to distinct sites. One taxon (OTU5) from the phylum Crenarchaeota was observed in all sites except Broder.

Permutational multivariate analyses were performed on the > 1% microbial community across site, depth and metal gradient; and it was determined that the structure of microbial communities was significantly difference across the 11 peatlands and over the metal gradient with $Pr(>F) < 0.05$ (Table 3.10). Conversely, there was no significant difference in the microbial community structure by depth across the study sites ($Pr(>F) > 0.05$) (Table 3.10). The microbial community structure across multiple fixed variables (simultaneous testing) was found to be significant for the interaction of any of the three variables (Table 3.10).

Permutational multivariate analyses were conducted for the microbial community against the chemical and physical properties of the sites, and community structure was significantly correlated with Al, Ni, Ca, pH and temperature (Table 3.11). The microbial species showed high significance with Ca and pH with Pr(>F) less than 0.05 and 0.001 respectively (Table 3.11).

PCA was also used to observe the difference in the environmental properties and microbial species distribution across 11 sites over three depths (10-20 cm, 30-40 cm and 60-70 cm), with 28.4% of the variability being explained with the first two axes (Figure 3.26). The first axis represented the physical and environmental factors, with positive loadings for pH, temperature, von Post and moisture, and the second axis represented the smelting deposition across the sites (Al, Cu, Ni). Along the second principal component axis, we see the surface samples (10-20 cm depth) clustering together with positive loadings for all sites, except for Ashigami and Cartier1. The sites at this depth were correlated with the smelting deposition gradient. There was clustering of the samples at the 30-40 cm and 60-70 cm depths along the first principal component axis with positive loadings for all sites at these two depths, except for Cartier1 and Cartier2. This indicated that the samples at these two depths were most affected by physical and environmental factors versus metals (Figure 3.26).

**Table 3.9**- Indicator species significantly ($p$-value < 0.05) associated with a specific indicator description, where indicator description represents site, depth, or metal grouping (from high-low metal). Indicator species $p$-value represents the probability of obtaining as high an indicator value as observed over 1000 permutations.

| OTU# | Indicator Description | Class | Family | Genus | Indicator species $p$-value |
|---|---|---|---|---|---|
| OTU18 | Site | Acidobacteriia | Acidobacteriaceae | --- | 0.021* |
| OTU19 | Site | Pedosphaerae | Ellin515 | --- | 0.019* |
| OTU16 | Site | Alphaproteobacteria | Bradyrhizobiaceae | Bradyrhizobium | 0.024* |
| OTU2 | Site | Deltaproteobacteria | Syntrophaceae | Desulfobacca | 0.019* |
| OTU35 | Site | Deltaproteobacteria | Syntrophaceae | Syntrophus | 0.018* |
| OTU15 | Depth | Deltaproteobacteria | Syntrophobacteraceae | --- | 0.006** |
| OTU7 | Depth | Acidobacteriia | Koribacteraceae | --- | 0.015* |
| OTU7 | Metal | Acidobacteriia | Koribacteraceae | --- | 0.040* |
| OTU1 | Metal | TM1 | --- | --- | 0.053. |
| OTU20 | Metal | Phycispaerae | --- | --- | 0.051. |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

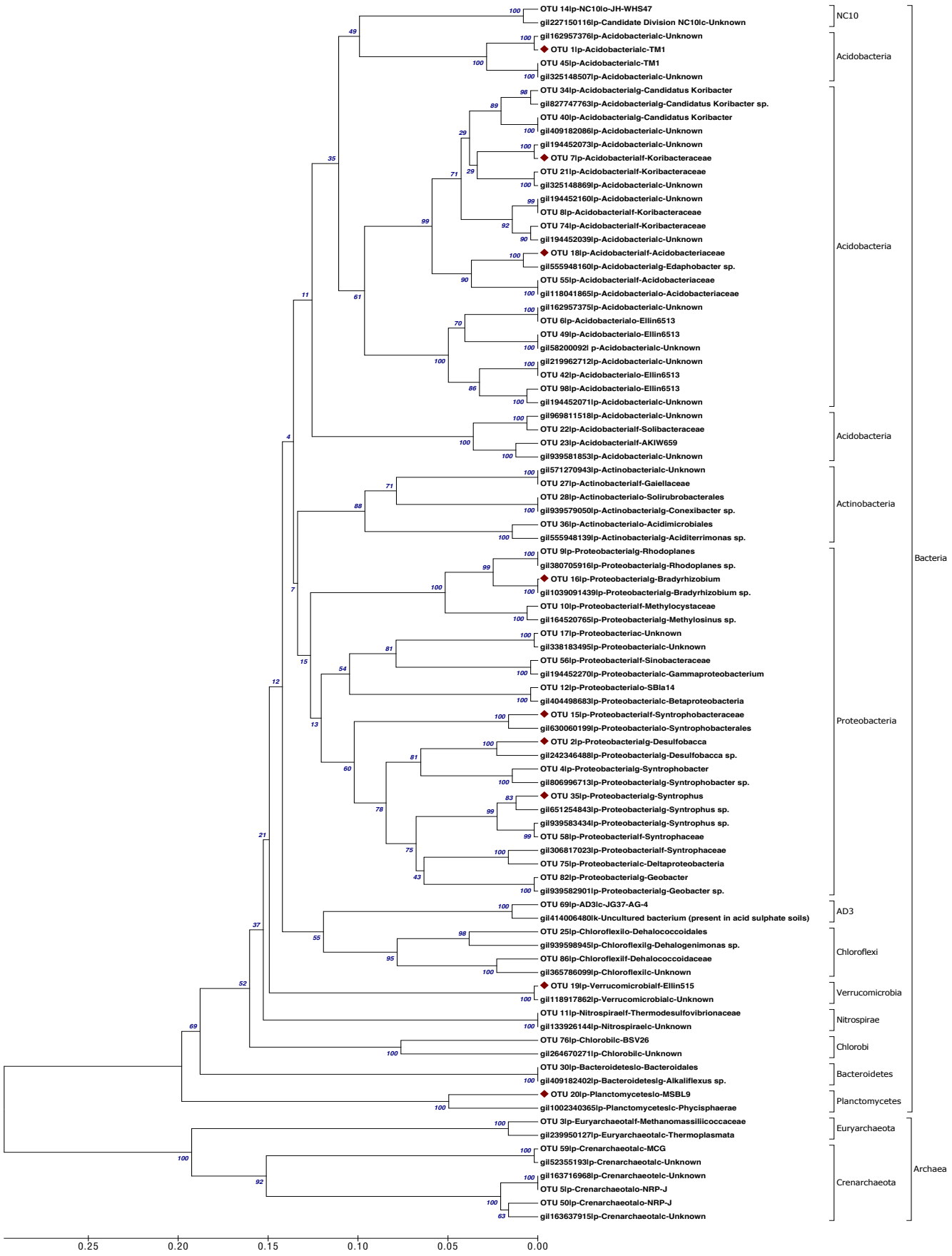

OTU 14|p-NC10|o-JH-WHS47
gil227150116|p-Candidate Division NC10|c-Unknown
gil162957376|p-Acidobacteria|c-Unknown
OTU 1|p-Acidobacteria|c-TM1
OTU 45|p-Acidobacteria|c-TM1
gil325148507|p-Acidobacteria|c-Unknown
OTU 34|p-Acidobacteria|g-Candidatus Koribacter
gil827747763|p-Acidobacteria|g-Candidatus Koribacter sp.
OTU 40|p-Acidobacteria|g-Candidatus Koribacter
gil409182086|p-Acidobacteria|c-Unknown
gil194452073|p-Acidobacteria|c-Unknown
OTU 7|p-Acidobacteria|f-Koribacteraceae
OTU 21|p-Acidobacteria|f-Koribacteraceae
gil325148869|p-Acidobacteria|c-Unknown
gil194452160|p-Acidobacteria|c-Unknown
OTU 8|p-Acidobacteria|f-Koribacteraceae
OTU 74|p-Acidobacteria|f-Koribacteraceae
gil194452039|p-Acidobacteria|c-Unknown
OTU 18|p-Acidobacteria|f-Acidobacteriaceae
gil555948160|p-Acidobacteria|g-Edaphobacter sp.
OTU 55|p-Acidobacteria|f-Acidobacteriaceae
gil118041865|p-Acidobacteria|o-Acidobacteriaceae
gil162957375|p-Acidobacteria|c-Unknown
OTU 6|p-Acidobacteria|o-Ellin6513
OTU 49|p-Acidobacteria|o-Ellin6513
gil58200092| p-Acidobacteria|c-Unknown
gil219962712|p-Acidobacteria|c-Unknown
OTU 42|p-Acidobacteria|o-Ellin6513
OTU 98|p-Acidobacteria|o-Ellin6513
gil194452071|p-Acidobacteria|c-Unknown
gil969811518|p-Acidobacteria|c-Unknown
OTU 22|p-Acidobacteria|f-Solibacteraceae
OTU 23|p-Acidobacteria|f-AKIW659
gil939581853|p-Acidobacteria|c-Unknown
gil571270943|p-Actinobacteria|c-Unknown
OTU 27|p-Actinobacteria|f-Gaiellaceae
OTU 28|p-Actinobacteria|o-Solirubrobacterales
gil939579050|p-Actinobacteria|g-Conexibacter sp.
OTU 36|p-Actinobacteria|o-Acidimicrobiales
gil555948139|p-Actinobacteria|g-Aciditerrimonas sp.
OTU 9|p-Proteobacteria|g-Rhodoplanes
gil380705916|p-Proteobacteria|g-Rhodoplanes sp.
OTU 16|p-Proteobacteria|g-Bradyrhizobium
gil1039091439|p-Proteobacteria|g-Bradyrhizobium sp.
OTU 10|p-Proteobacteria|f-Methylocystaceae
gil164520765|p-Proteobacteria|g-Methylosinus sp.
OTU 17|p-Proteobacteriac-Unknown
gil338183495|p-Proteobacteria|c-Unknown
OTU 56|p-Proteobacteria|f-Sinobacteraceae
gil194452270|p-Proteobacteria|c-Gammaproteobacterium
OTU 12|p-Proteobacteria|o-SBla14
gil404498683|p-Proteobacteria|c-Betaproteobacteria
OTU 15|p-Proteobacteria|f-Syntrophobacteraceae
gil630060199|p-Proteobacteria|o-Syntrophobacterales
OTU 2|p-Proteobacteria|g-Desulfobacca
gil242346488|p-Proteobacteria|g-Desulfobacca sp.
OTU 4|p-Proteobacteria|g-Syntrophobacter
gil806996713|p-Proteobacteria|g-Syntrophobacter sp.
OTU 35|p-Proteobacteria|g-Syntrophus
gil651254843|p-Proteobacteria|g-Syntrophus sp.
gil939583434|p-Proteobacteria|g-Syntrophus sp.
OTU 58|p-Proteobacteria|f-Syntrophaceae
gil306817023|p-Proteobacteria|f-Syntrophaceae
OTU 75|p-Proteobacteria|c-Deltaproteobacteria
OTU 82|p-Proteobacteria|g-Geobacter
gil939582901|p-Proteobacteria|g-Geobacter sp.
OTU 69|p-AD3|c-JG37-AG-4
gil414006480|k-Uncultured bacterium (present in acid sulphate soils)
OTU 25|p-Chloroflexi|o-Dehalococcoidales
gil939598945|p-Chloroflexi|g-Dehalogenimonas sp.
OTU 86|p-Chloroflexi|f-Dehalococcoidaceae
gil365786099|p-Chloroflexi|c-Unknown
OTU 19|p-Verrucomicrobia|f-Ellin515
gil118917862|p-Verrucomicrobia|c-Unknown
OTU 11|p-Nitrospirae|f-Thermodesulfovibrionaceae
gil133926144|p-Nitrospirae|c-Unknown
OTU 76|p-Chlorobi|c-BSV26
gil264670271|p-Chlorobi|c-Unknown
OTU 30|p-Bacteroidetes|o-Bacteroidales
gil409182402|p-Bacteroidetes|g-Alkaliflexus sp.
OTU 20|p-Planctomycetes|o-MSBL9
gil1002340365|p-Planctomycetes|c-Phycisphaerae
OTU 3|p-Euryarchaeota|f-Methanomassiliicoccaceae
gil239950127|p-Euryarchaeota|c-Thermoplasmata
OTU 59|p-Crenarchaeota|c-MCG
gil523551939|p-Crenarchaeota|c-Unknown
gil163716968|p-Crenarchaeota|c-Unknown
OTU 5|p-Crenarchaeota|o-NRP-J
OTU 50|p-Crenarchaeota|o-NRP-J
gil163637915|p-Crenarchaeota|c-Unknown
NC10
Acidobacteria
Acidobacteria
Acidobacteria
Actinobacteria
Proteobacteria
AD3
Chloroflexi
Verrucomicrobia
Nitrospirae
Chlorobi
Bacteroidetes
Planctomycetes
Euryarchaeota
Crenarchaeota
Bacteria
Archaea
0.25
0.20
0.15
0.10
0.05
0.00

**Figure 3.27-** The evolutionary history was inferred using the UPGMA method. The sum of branch length = 4.77 is shown with bootstrap values indicating the strength of each node (500 replicates). The evolutionary distances were computed using the Tajima-Nei method. The analysis involved 90 nucleotide sequences. There were a total of 257 positions in the final data set. Highlighted organisms represent potential indicator species with significant *p*-value (*p*-value < 0.05). (|p=Phylum, |c= Class, |o=Order, |f=Family, |g=Genus).

**Table 3.10**- Permutational multivariate analysis on OTUs > 1% relative abundance, using Bray-Curtis distance matrices, to determine the significant difference between these OTU's versus levels of site, depth, description. Bray-Curtis was used to analysize variance over 49 permuations of the data. Significance was measured, where Pr(>F) represents the significant probability value associated with the F-value after permuations.

| **Variables** | **$R^2$** | **Pr (>F)** |
|---|---|---|
| Site | 0.601 | 0.001*** |
| Depth | 0.083 | 0.137 |
| Description | 0.170 | 0.001*** |
| Depth + Site | 0.083; 0.599 | 0.001***; 0.001*** |
| Depth + Description | 0.083; 0.171 | 0.060.; 0.001*** |
| Description + Site | 0.170; 0.431 | 0.001***; 0.001*** |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Depth + Site, Depth + Description, and Description + Site represent two fixed factors without interactions.

**Table 3.11**- Permutational multivariate analysis on OTUs > 1% relative abundance, using Bray-Curtis distance matrices, to determine the significant difference between these OTU's versus the studied chemical and physical factors. Bray-Curtis was used to analysize variance over 999 permuations of the data. Significance was measured, where Pr(>F) represents the significant probability value associated with the F-value after permuations.

| **Variables** | **$R^2$** | **Pr(>F)** |
|---|---|---|
| Al | 0.051 | 0.056. |
| Cu | 0.041 | 0.163 |
| Ni | 0.049 | 0.061. |
| K | 0.037 | 0.305 |
| Ca | 0.053 | 0.045* |
| pH | 0.101 | 0.001*** |
| Temperature | 0.049 | 0.074. |
| Von Post score | 0.030 | 0.511 |
| Moisture score | 0.032 | 0.488 |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

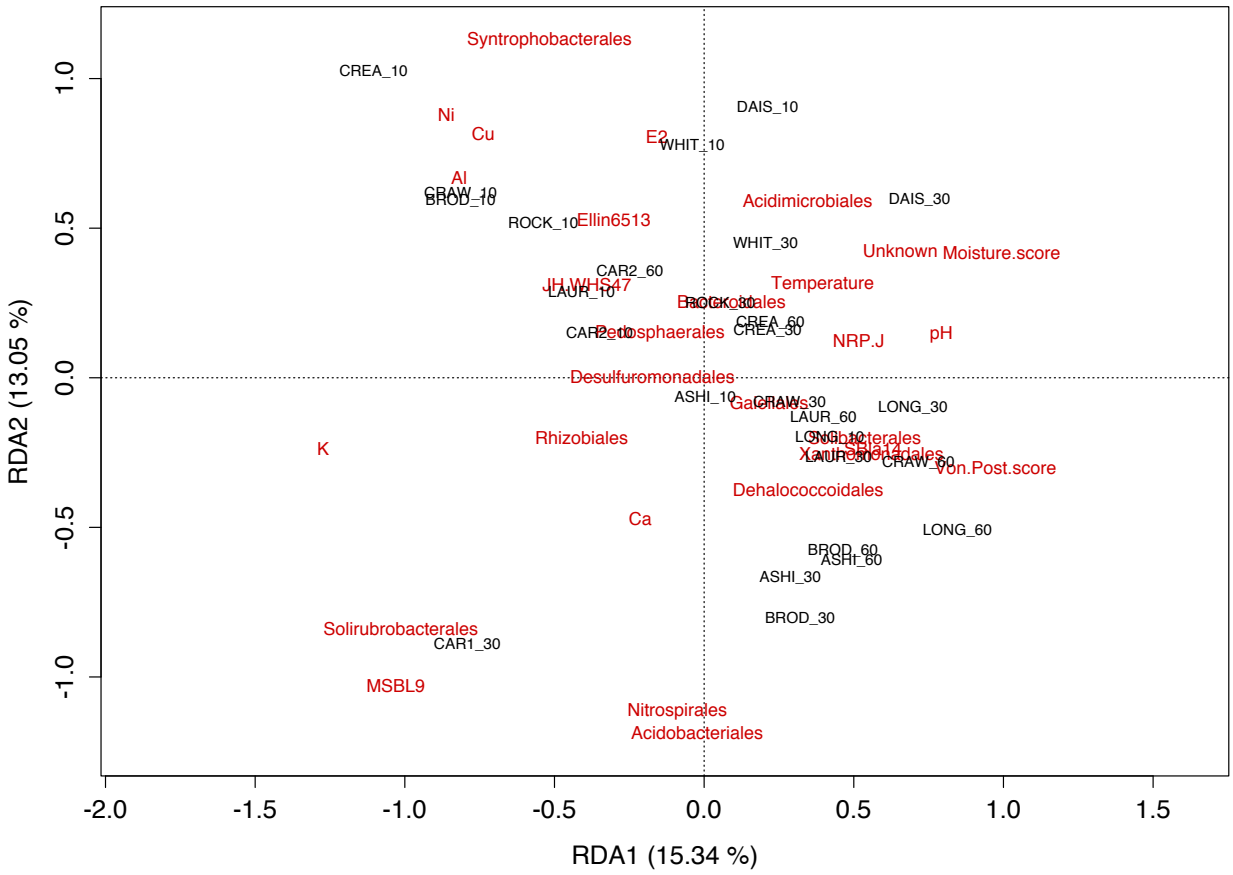



**Figure 3.28-** Principal component analysis for environmental variables versus the abundant (> 1%) microbial species over four depth increments (10-20 cm, 30-40 cm and 60-70 cm depth) across 11 study sites. PC1 and PC2 explained 15.34% and 13.05% proportion of variance respectively.

### 3.3.3 The Impact of Highly Abundant Taxa and Indicator Species

After examining the > 1% abundance data set using the depth heat-maps (Figure 3.22-Figure 3.24), it was determined that there were some OTUs that were highly abundant across site and depth. A taxonomic subset containing OTUs > 2% abundance of the original OTU data set was created in order to test the effects of environmental drivers on these highly abundant OTUs across the metal gradient. Nineteen OTUs were obtained from the > 2% analysis which represented 27.8% of the total OTU abundance from the original 9166 OTUs, and also 69.3% of the total abundance for the > 1% OTUs. All the indicator species (Table 3.9) overlapped with the > 2% subset, with the exception of OTU 19 and OTU 35; which were still included for the purpose of analyzing indicator species and highly abundant OTUs across the metal gradient.

Univariate permutational analyses were conducted on the 21 OTUs to determine the significant difference between these highly abundant taxa and environmental factors. Of the 21 OTUs, 4 species were significant ($F < 0.05$) for Cu, 6 for Ni and 8 for Al, indicating that metals represent a major factor influencing microbial taxa (Table 3.14). There was a total of 11 OTUs with significant Pr(>F) values ($F < 0.05$) for Ca, and 2 for K, indicating that mineral factors (especially Ca) greatly influenced microbial taxa across the study sites. Temperature represented one of the major environmental factors affecting the 21 key OTUs, with 9 OTUs having significant Pr(>F) values ($F < 0.05$), while von Post played a lesser role as an influencing factor with 5 OTUs showing significance difference across the samples. pH was the major driving factor of the nine environmental factors, influencing microbial taxa across the study samples, with 15 of the 21 OTUs having significance values ($F < 0.05$) (Table 3.14).

**Table 3.12**- Univariate permuational analysis on 21 key OTU's to determine the significant difference between these OTU's versus studied chemical and physical factors. Permutations of 499 was used to analysize the variance of the data for each key OTU. Significance was measured using F-value and Pr(>F), where Pr(>F) represents the significant probability value associated with the F-value after permuations.

| **Variables** | **OTU1** | | **OTU10** | | **OTU11** | | **OTU12** | | **OTU14** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** |
| Cu | 4.48 | 0.034* | 19.8 | 0.004** | 2.77 | 0.06. | 0.458 | 0.488 | 0.660 | 0.418 |
| Ni | 6.83 | 0.018* | 4.38 | 0.046* | 0.565 | 0.470 | 0.871 | 0.308 | 0.783 | 0.368 |
| Ca | 0.187 | 0.674 | 10.9 | 0.002** | 4.56 | 0.048* | 26.0 | 0.002** | 28.9 | 0.002** |
| K | 6.29 | 0.026* | 9.00e-04 | 0.984 | 1.67 | 0.206 | 0.430 | 0.452 | 0.221 | 0.656 |
| Al | 0.900 | 0.346 | 9.28 | 0.014* | 3.92 | 0.062. | 6.38 | 0.016* | 7.28 | 0.014* |
| Temperature | 22.5 | 0.002** | 0.270 | 0.584 | 3.39 | 0.062. | 0.005 | 0.948 | 0.106 | 0.708 |
| von Post | 1.67 | 0.226 | 3.33 | 0.064. | 0.204 | 0.596 | 4.96 | 0.026* | 8.11 | 0.004** |
| Moisture | 2.20 | 0.064. | 0.379 | 0.524 | 1.37 | 0.282 | 0.416 | 0.442 | 0.539 | 0.768 |
| pH | 26.8 | 0.002** | 21.5 | 0.002** | 0.985 | 0.328 | 12.3 | 0.004** | 16.3 | 0.002** |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

**Table 3.12 continued**- Univariate permuational analysis on 21 key OTU's to determine the significant difference between these OTU's versus studied chemical and physical factors. Permutations of 499 was used to analysize the variance of the data for each key OTU. Significance was measured using F-value and Pr(>F), where Pr(>F) represents the significant probability value associated with the F-value after permuations.

| **Variables** | **OTU15** | | **OTU16** | | **OTU18** | | **OTU2** | | **OTU3** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** |
| Cu | 21.8 | 0.006** | 0.338 | 0.532 | 1.48 | 0.176 | 2.96 | 0.08. | 0.191 | 0.684 |
| Ni | 9.11 | 0.016* | 0.098 | 0.772 | 2.77 | 0.07. | 3.64 | 0.06. | 0.371 | 0.544 |
| Ca | 0.795 | 0.378 | 7.63 | 0.012* | 5.25 | 0.022* | 0.607 | 0.438 | 1.90 | 0.174 |
| K | 1.70 | 0.09. | 0.282 | 0.534 | 1.81 | 0.184 | 3.08 | 0.082. | 0.307 | 0.596 |
| Al | 9.14 | 0.018* | 26.7 | 0.004** | 0.552 | 0.432 | 6.18 | 0.028* | 0.469 | 0.500 |
| Temperature | 0.888 | 0.328 | 0.543 | 0.456 | 0.053 | 0.808 | 10.3 | 0.004** | 0.624 | 0.420 |
| von Post | 0.941 | 0.372 | 1.05 | 0.300 | 0.038 | 0.846 | 0.015 | 0.904 | 0.013 | 0.896 |
| Moisture | 6.52 | 0.058. | 0.326 | 0.402 | 0.746 | 0.426 | 2.24 | 0.106 | 5.00 e-04 | 0.984 |
| pH | 5.98 | 0.008** | 0.945 | 0.366 | 0.003 | 0.958 | 23.3 | 0.002** | 10.1 | 0.004** |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

**Table 3.12 continued**- Univariate permuational analysis on 21 key OTU's to determine the significant difference between these OTU's versus studied chemical and physical factors. Permutations of 499 was used to analysize the variance of the data for each key OTU. Significance was measured using F-value and Pr(>F), where Pr(>F) represents the significant probability value associated with the F-value after permuations.

| **Variables** | **OTU34** | | **OTU4** | | **OTU5** | | **OTU56** | | **OTU6** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** |
| Cu | 13.7 | 0.012* | 0.135 | 0.680 | 0.300 | 0.592 | 0.009 | 0.93 | 2.88 | 0.086. |
| Ni | 3.78 | 0.048* | 0.571 | 0.460 | 0.086 | 0.796 | 1.03 | 0.308 | 0.680 | 0.410 |
| Ca | 2.63 | 0.114 | 0.721 | 0.392 | 3.94 | 0.050* | 3.39 | 0.076. | 5.03 | 0.02* |
| K | 0.187 | 0.712 | 0.566 | 0.458 | 3.87 | 0.042* | 0.779 | 0.314 | 1.16 | 0.272 |
| Al | 0.002 | 0.968 | 2.31 | 0.12 | 2.25 | 0.174 | 0.520 | 0.524 | 1.24 | 0.256 |
| Temperature | 6.91 | 0.018* | 17.0 | 0.002** | 0.127 | 0.726 | 4.03 | 0.04* | 9.16 | 0.002** |
| von Post | 1.31 | 0.310 | 0.146 | 0.680 | 8.00e-04 | 0.970 | 0.070 | 0.762 | 0.332 | 0.596 |
| Moisture | 0.116 | 0.918 | 0.549 | 0.400 | 0.056 | 0.824 | 6.25e-06 | 0.514 | 0.085 | 0.794 |
| pH | 3.86 | 0.06. | 48.5 | 0.002** | 4.22 | 0.056. | 13.7 | 0.002** | 15.8 | 0.002** |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

**Table 3.12 continued**- Univariate permuational analysis on 21 key OTU's to determine the significant difference between these OTU's versus studied chemical and physical factors. Permutations of 499 was used to analysize the variance of the data for each key OTU. Significance was measured using F-value and Pr(>F), where Pr(>F) represents the significant probability value associated with the F-value after permuations.

| **Variables** | **OTU7** | | **OTU76** | | **OTU8** | | **OTU9** | | **OTU19** | | **OTU35** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** | **F-value** | **Pr(>F)** |
| Cu | 2.25 | 0.126 | 2.40 | 0.096. | 1.22 | 0.260 | 3.62 | 0.058. | 0.717 | 0.436 | 0.732 | 0.358 |
| Ni | 1.37 | 0.210 | 4.65 | 0.030* | 1.63 | 0.188 | 5.65 | 0.024* | 0.756 | 0.398 | 1.04 | 0.304 |
| Ca | 0.164 | 0.690 | 2.19 | 0.128 | 3.06 | 0.07. | 5.05 | 0.028* | 29.1 | 0.002** | 22.7 | 0.002** |
| K | 0.435 | 0.454 | 2.57 | 0.096. | 2.19 | 0.128 | 0.909 | 0.348 | 0.179 | 0.680 | 0.291 | 0.588 |
| Al | 0.589 | 0.408 | 1.50 | 0.236 | 1.62 | 0.220 | 1.27 | 0.284 | 7.48 | 0.018* | 6.82 | 0.016* |
| Temperature | 11.6 | 0.006** | 0.92 | 0.354 | 7.25 | 0.006** | 7.23 | 0.006** | 0.118 | 0.752 | 0.004 | 0.938 |
| von Post | 0.657 | 0.488 | 11.1 | 0.002** | 3.40 | 0.08. | 1.24 | 0.270 | 9.37 | 0.004** | 8.73 | 0.006** |
| Moisture | 1.03 | 0.096. | 0.721 | 0.608 | 0.963 | 0.328 | 0.000 | 0.998 | 0.577 | 0.700 | 0.531 | 0.610 |
| pH | 10.9 | 0.002** | 9.12 | 0.004** | 51.5 | 0.002** | 2.38 | 0.146 | 17.6 | 0.002** | 16.1 | 0.002** |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

## 3.4 The Relationship between Key Microbial Taxa and Peatland Ecology

Across the metal gradient, from low-impacted to high impact, we see a difference in OTU composition and abundance but there is also a difference in the plant communities across this gradient. The low-impacted sites had the lowest Cu and Ni concentrations and the lowest pH (< 3.53) and von Post (< 5.08) compared to the other sites (Table 3.13). These also had the lowest shrub and sedge coverage, with the exception of *Ledum groenlandicum* and *Eriophorum* sp. occupying a third of the area in Cartier1 and Cartier2 respectively (Table 3.13). The intermediate-low impacted sites had relatively low Cu and Ni concentrations along with von Post < 5.75 and pH between 4.31-5.24, and they also had generally low shrub and sedge coverage, except for *Chamaedaphne calyculata,* which appeared in higher abundance (Table 3.13).

The intermediate-high impacted sites had relatively high Cu and Ni, with von Post > 6.0 and pH between 4.16-5.79. They also had relatively high sedge cover especially Daisy I with 80% *Eriophorum* sp. cover. There was a range of % cover for shrubs between the sites where *Chamaedaphne calyculata* had the highest cover among the sites, with the exception of Daisy where it was absent (Table 3.13). *Ledum groenlandicum* and *Kalmia* sp. were absent except for Crowley (6.7% and 11.7% respectively) (Table 3.13). The high-impacted sites had the highest Cu and Ni concentrations, with von Post > 5.0 and pH between 3.74-6.18. There was also low sedge coverage but a high shrub cover (*Chamaedaphne calyculata*) except for at Whitson with 1.71% *Chamaedaphne calyculata* (Table 3.13).

A decreasing trend was observed between *Sphagnum* moss richness and both Cu and Ni concentrations across the study sites (Figure 3.27). *Sphagnum* moss richness was highest in the sites with the lowest Cu and Ni concentrations (low and intermediate-low impacted sites), and it was lowest in the sites with the highest Cu and Ni concentrations (high and intermediate-high impacted sites).

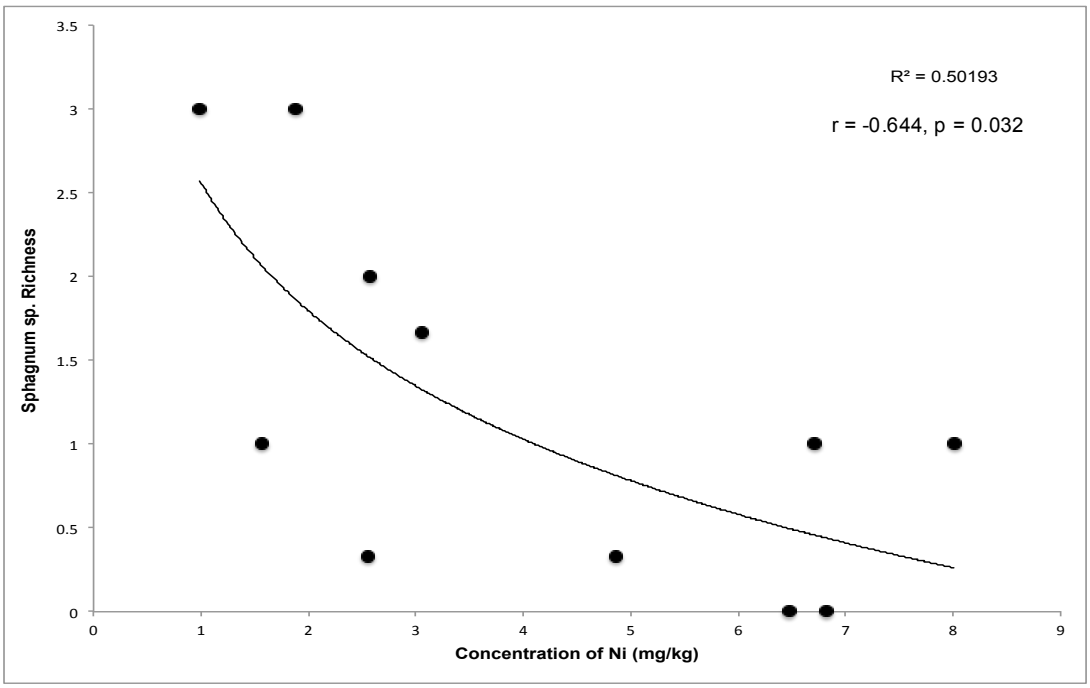
Sites were differentiated using redundancy analysis (RDA) for the plant species; the cumulative proportion of variance for both axes was 61% (Figure 3.28). The low and intermediate-low impacted sites were influenced by moisture and plant species including moss, shrubs and herbs (*Hypericum*, *Drosera*, and *Sarracenia* (Figure 3.28). The intermediate- high and high-impacted sites were influenced by chemistry and pH, along with plant species including shrubs, sedges, grasses (*Glyceria*), rushes (*Juncus*) (Figure 3.28). Additionally, a Mantel test was conducted examining the differences between plant species and environmental variables, confirming that the differences between the two were significant (p=0.05, Figure 3.28).

Sites were then differentiated using redundancy analysis (RDA) for the plant species and microbial species combined, with a cumulative proportion of variance of 61.2% (Figure 3.29). Cartier1 and Rockcut had similar variances and were dominated by *Myrica gale* and microbial species such as MSBL9 (Figure 3.29). Cartier2 was dominated by plant species such as *Vaccinium, Kalmia* and *Sarracenia* and microbial species such as Pedosphaerales (Figure 3.29). Ashigami was dominated by several plant species including *Hypericum, Typha, Drosera* and microbial species including JH-WHS47. JH-WHS47 and *Hypericum* and *Sphagnum* also dominated Whitson. Daisy-I had an abundance of *Eriophorum* and *Glyceria* and also microbial species including Syntrophobacterales. Laurentian, Clearwater and Crowley all had similar variances and were dominated by *Chamaedaphne*, *Ledum*, *Maianthemum trifolium* and *Salix pedicellaris* and microbial species including SBla14. Microbes such as Acidobacteriales and Rhizobiales dominated Long Lake, along with plant species including *Carex*. Lastly, Broder had an abundance of *Alnus incana, Juncus* and *Dulichium arundinaceum* and also microbial species including Nitrospirales and Solibacterales (Figure 3.29). A Mantel test was conducted examining the differences between the plant species and the microbial species but when considering both the plant and microbial communities, it was not possible to distinguish the sites as with microbial data alone (Figure 3.29).

**Table 3.13-** Environmental properties and percent cover of 6 key vascular and non-vascular peatland plant species over the 11 chosen sites; categorizing the sites from low metal impacted to high metal impacted. Error is represented as standard error of the mean (SE).

| **Sites** | **Mean [Cu] (mg/kg)** | **Mean [Ni] (mg/kg)** | **Mean pH** | **Mean von Post** | **% Cover *Chamaedaphne calyculata*** | **% Cover *Ledum groenlandicum*** | **% Cover *Kalmia* sp.** | **% Cover *Eriophorum* sp.** | **% Cover *Carex* sp.** | **% Cover *Sphagnum* sp.** |
|---|---|---|---|---|---|---|---|---|---|---|
| Cartier1 | 0.190 +/-0.04 | 0.992 +/-0.17 | 3.51 +/-0.01 | 5.08 +/-0.56 | 11.7 | 36.7 | 6.70 | 5.00 | 0.00 | 83.3 |
| Cartier2 | 0.275 +/-0.05 | 1.88 +/-0.31 | 3.53 +/-0.03 | 4.08 +/-0.31 | 6.70 | 0.00 | 3.30 | 30.0 | 0.00 | 70.0 |
| Ashigami | 0.232 +/-0.07 | 2.58 +/-0.56 | 5.17 +/-0.22 | 5.25 +/-0.60 | 21.7 | 0.00 | 1.70 | 1.70 | 3.30 | 38.3 |
| Rockcut | 0.388 +/-0.13 | 3.06 +/-1.03 | 4.31 +/-0.07 | 5.40 +/-0.60 | 5.00 | 0.00 | 0.00 | 3.30 | 0.00 | 66.7 |
| Broder | 0.744 +/-0.22 | 6.71 +/-2.05 | 5.24 +/-0.12 | 5.75 +/-0.62 | 40.0 | 3.30 | 0.00 | 0.300 | 3.30 | 10.7 |
| Long Lake | 0.968 +/-0.28 | 1.57 +/-0.33 | 5.79 +/-0.14 | 6.17 +/-0.61 | 23.3 | 0.00 | 0.00 | 0.00 | 46.7 | 15.0 |
| Crowley | 1.36 +/-0.43 | 4.87 +/-1.47 | 4.16 +/-0.18 | 6.67 +/-0.50 | 63.3 | 6.70 | 11.7 | 13.3 | 3.30 | 0.00 |
| Daisy I | 1.61 +/-0.50 | 2.55 +/-0.45 | 5.15 +/-0.24 | 6.44 +/-0.24 | 0.00 | 0.00 | 0.00 | 80.0 | 0.00 | 0.00 |
| Laurentian | 2.37 +/-0.78 | 6.82 +/-2.10 | 4.80 +/-0.13 | 6.83 +/-0.37 | 50.0 | 0.00 | 0.00 | 10.0 | 1.70 | 0.00 |
| Clearwater | 2.58 +/-0.93 | 6.48 +/-2.00 | 3.74 +/-0.03 | 5.83 +/-0.46 | 76.7 | 0.00 | 0.00 | 3.30 | 0.00 | 0.00 |
| Whitson | 2.61 +/-0.97 | 8.01 +/-2.69 | 6.19 +/-0.22 | 5.00 +/-0.50 | 1.70 | 0.00 | 0.00 | 0.00 | 40.0 | 41.7 |

(A)




(B)

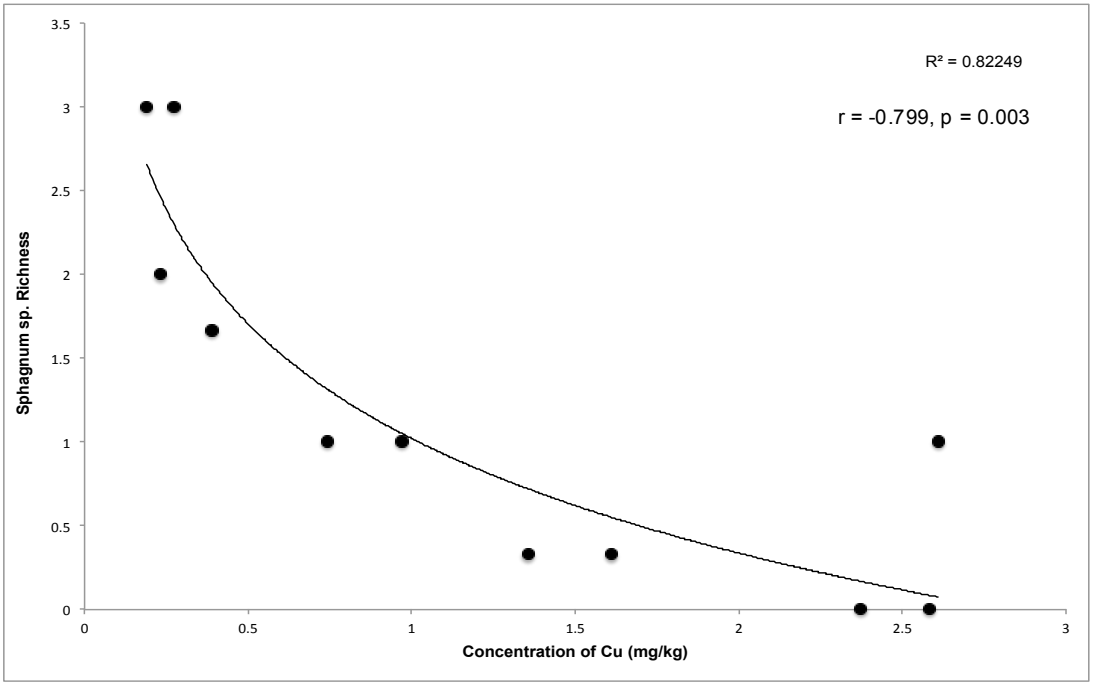



**Figure 3.29-** The relationship between *Sphagnum* moss richness and Ni (graph A) and Cu (graph B) concentrations across the 11 study sites. The Pearson correlation is represented by r and the correlation significance is represented by *p*.

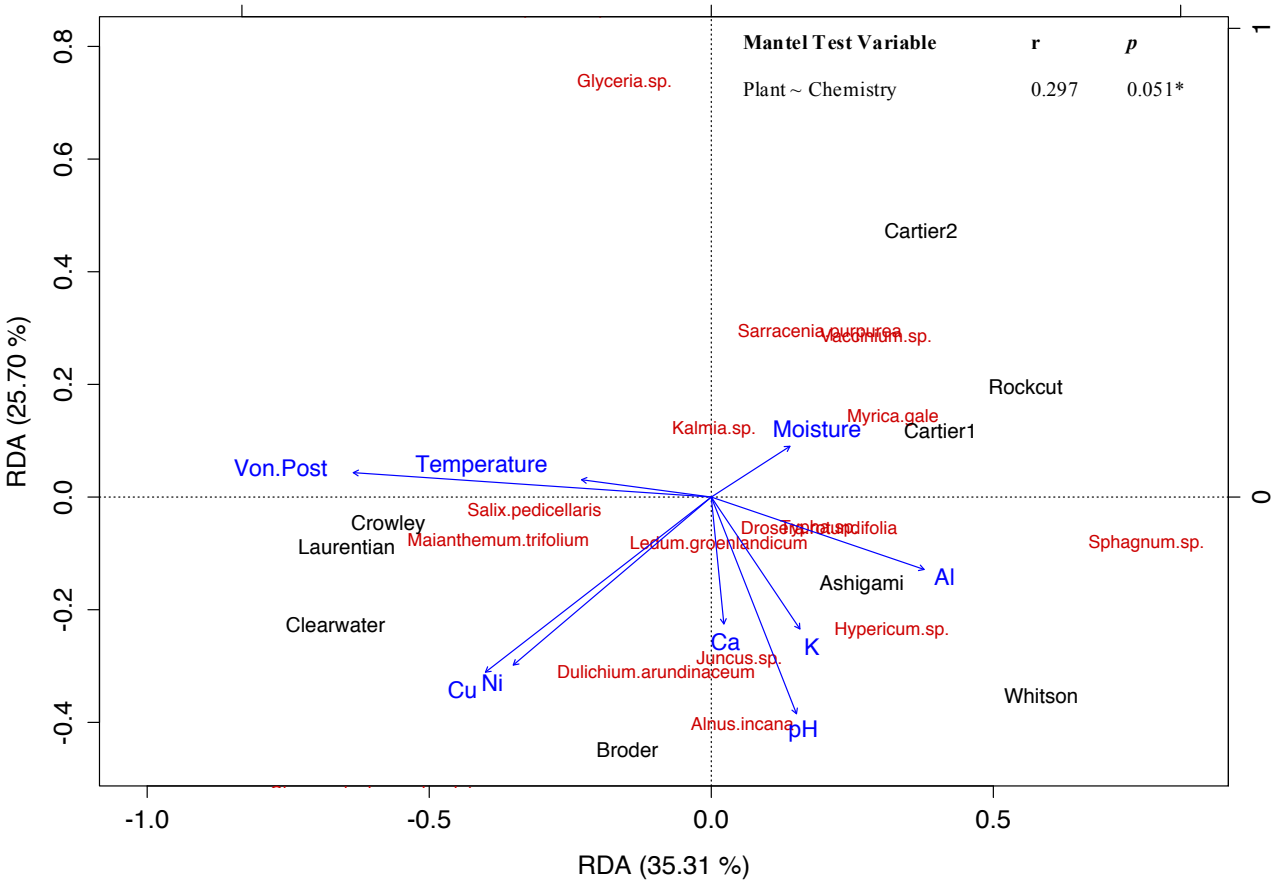



**Figure 3.30-** Redundancy analysis for environmental variables in each sample and how these variables affect the plant community present in each of these sites. The plant community percent cover was transformed using Hellinger transformation. RDA1 and RDA2 explained 35.31% and 25.70% proportion of variance respectively. A Mantel test was conducted on the Euclidean matrix for the plant species versus the Euclidean matrix for the environmental variables using 9999 permutations.

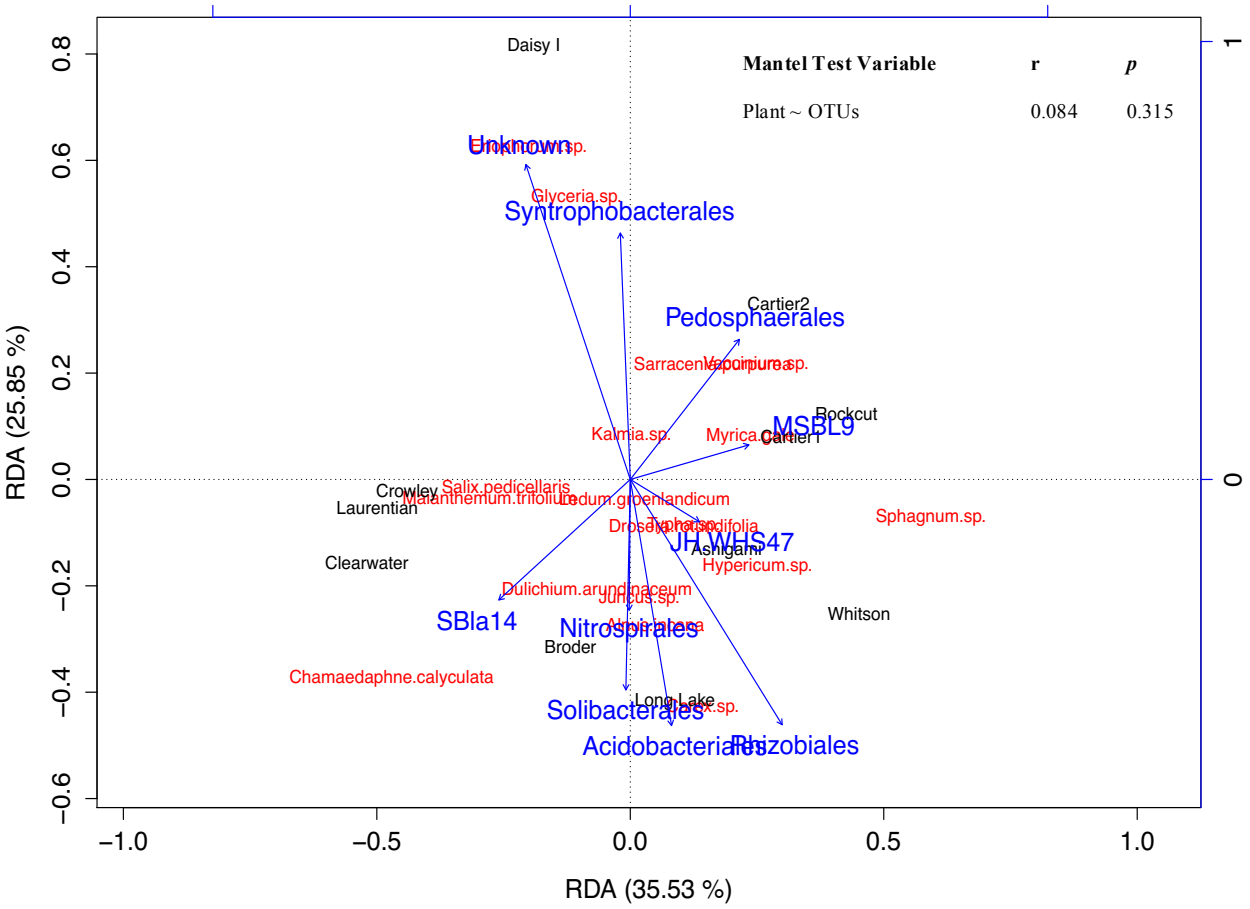



**Figure 3.31-** Redundancy analysis for environmental variables in each sample and how these variables affect plant community and key OTUs in each of these sites. The plant community percent cover was transformed using Hellinger transformation and analyzed against OTU abundance. RDA1 and RDA2 explained 35.53% and 25.85% proportion of variance respectively. A Mantel test was conducted on the Euclidean matrix for the plant species versus the Euclidean matrix for the OTUs using 9999 permutations.

## 3.5 Microbial Function and Enzymatic Analysis

### 3.5.1 Microbial Function: Hydrolases

One-way ANOVA analyses were conducted to examine the effects of microbial enzymatic activity for carbon, nitrogen, sulphur and phosphorus acquiring microbes across a gradient of sites from low-impacted to high-impacted. The simple main effects analysis showed statistical significance between sites at specific depths for high, intermediate high, intermediate low and low-impacted sites. The observed mean glucosidase activity at the 0-10 cm depth was lowest in the least impacted sites, and was generally higher in the high-impacted sites such as Whitson (high-impacted), which had the highest mean activity of the 11 sites (Figure 3.30). The results of the S.N.K. post-hoc test showed that the two low-impacted sites (Cartier 1 and Cartier 2) were statistically different from Whitson (Figure 3.30). Mean extracellular glucosaminidase activity at 30-40 cm showed that the highest mean activity occurred in the intermediate low and low-impacted sites and the lowest activity occurred in the high and intermediate-high impacted sites, with the exception of Daisy I (Figure 3.30). Cartier 2 (least impacted) had the highest glucosaminidase activity and Clearwater (high-impacted) had the lowest glucosaminidase activity (Figure 3.30). There were no trends for the mean phosphatase activity at 0-10 cm (Figure 3.30) and arylsulphatase activity at 30-40 cm (Figure 3.30) across the sites over the impact gradients. The one-way ANOVA conducted on the mean P:S ratio across the sites showed no trends at the 0-10 cm depth (Figure 3.31). Crowley and Rockcut showed no significant difference from each other but they were significantly different from the other 9 sites (Figure 3.31). These two sites had the highest P: S ratio of the 11 sites (Figure 3.31) despite having different metal impacts and being different distances away from the smelters. Analyses were conducted on the N: P ratio across the sites at the 30-40 cm revealed that the least impacted sites (Cartier 1 and Cartier 2) had the highest mean N: P activity of the 11 sites, with Cartier 2 having the highest

mean activity (Figure 3.31). The overall N: P ratio was relatively low across all the sites except for the low-impacted sites (Figure 3.31).

A two-way ANOVA analysis and Post-hoc (S.N.K) were conducted to examine the interaction between site and depth on the microbial activity of carbon, nitrogen, sulphur and phosphorus acquiring microbes. There was a statistically significant interaction between the effects of depth horizontally on enzyme activity for the metal gradient from high, intermediate high, intermediate low and low-impacted sites. There was a consistent decreasing trend from the 0-10 cm depth to the 60-70 cm depth for the all three enzyme tests that were conducted (Figure 3.32). In general, the mean phosphatase activity was higher than the mean glucosidase and glucosaminidase activities for all four depths studied (Figure 3.32). The two surface depths (0-10 cm and 10-20 cm) showed no significant differences in mean enzymatic activities (except for mean phosphatase), while both deep depths (30-40 cm and 60-70 cm) showed significant differences (Figure 3.32). Mean phosphatase activities decreased from 6.16 (+/- 0.77) at the 0 cm depth to 0.65 (+/-0.13) at the 60 cm depth, while mean glucosidase activities decreased from 2.41 (+/- 0.41) at the 0 cm depth to 0.16 (+/- 0.02) at the 60 depth, and lastly glucosaminidase activities decreased from 1.39 (+/- 0.25) at the 0 cm depth to 0.07 (+/- 0.02) at the 60 cm depth (Figure 3.32).

Analyses conducted on the enzyme ratios showed a consistent decreasing trend from the surface depth to the deep peat depths (Figure 3.33). Concerning C:S and N:S, these ratios showed similar trends where the two surface depths (0-10, and 10-20 cm) were similar, but were significantly different in activity from the deep peat (20-30, and 60-70 cm) depths. Additionally, the two deep peat depths were significantly different from each other (Figure 3.33). For N:P ratios the two surface depths showed no significant difference between each other but they were significantly different from the two deep peat depths; similarly, the deep peat depths were not significantly different from each other (Figure 3.33). Lastly, P:S ratios showed that all four depths as being significantly different from each other (Figure 3.33).

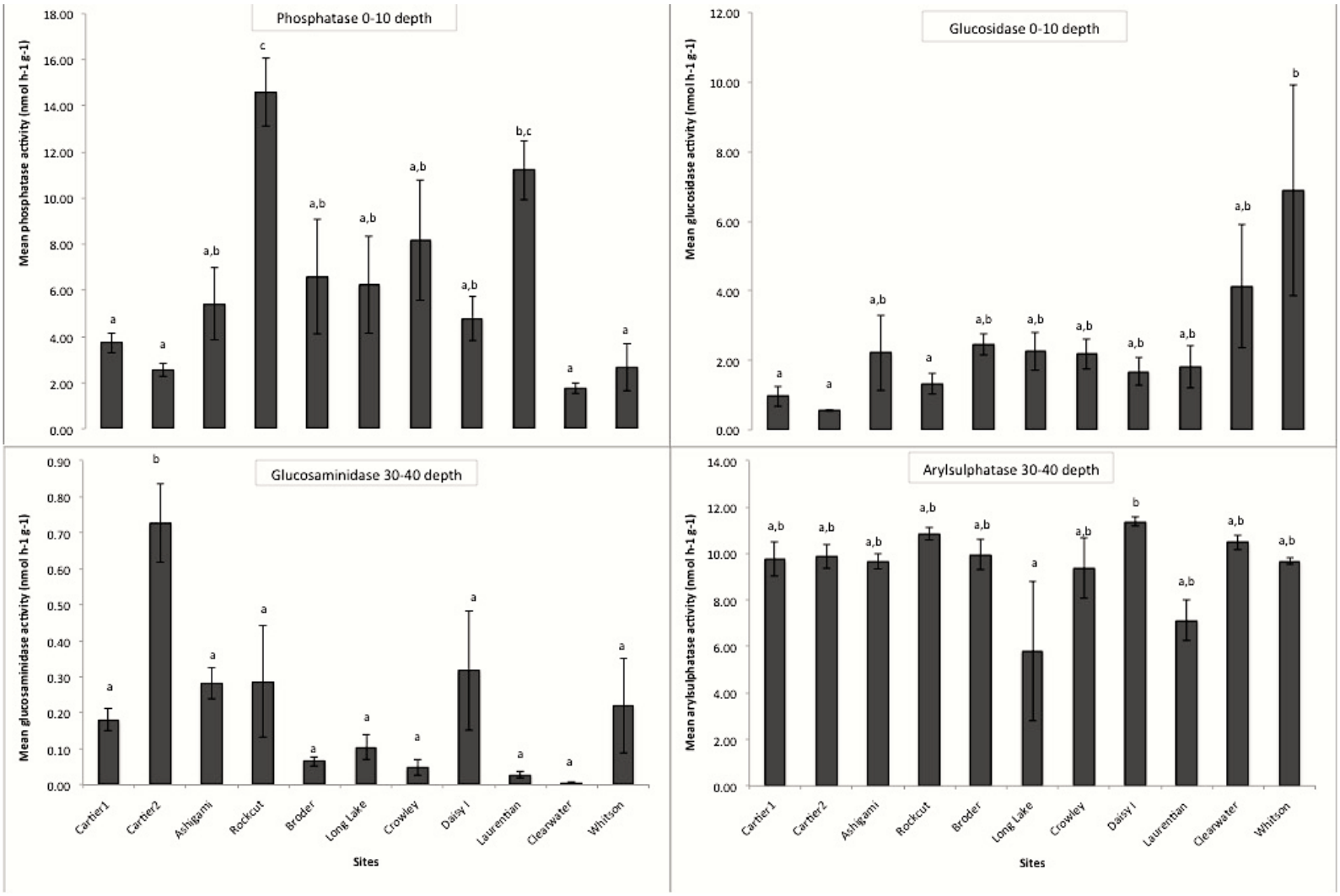



**Figure 3.32-** Mean extracellular activity of microbial communities across 11 study sites for phosphatase 0-10 cm (top left), glucosidase 0-10 cm (top right), glucosaminidase 30-40 cm (bottom left) and arylsulphatase 30-40 cm (bottom right), over a metal gradient. Error bars represent standard error and the letters represent the results of ANOVA tests, where shared letter indicate no significant differences between the sites.

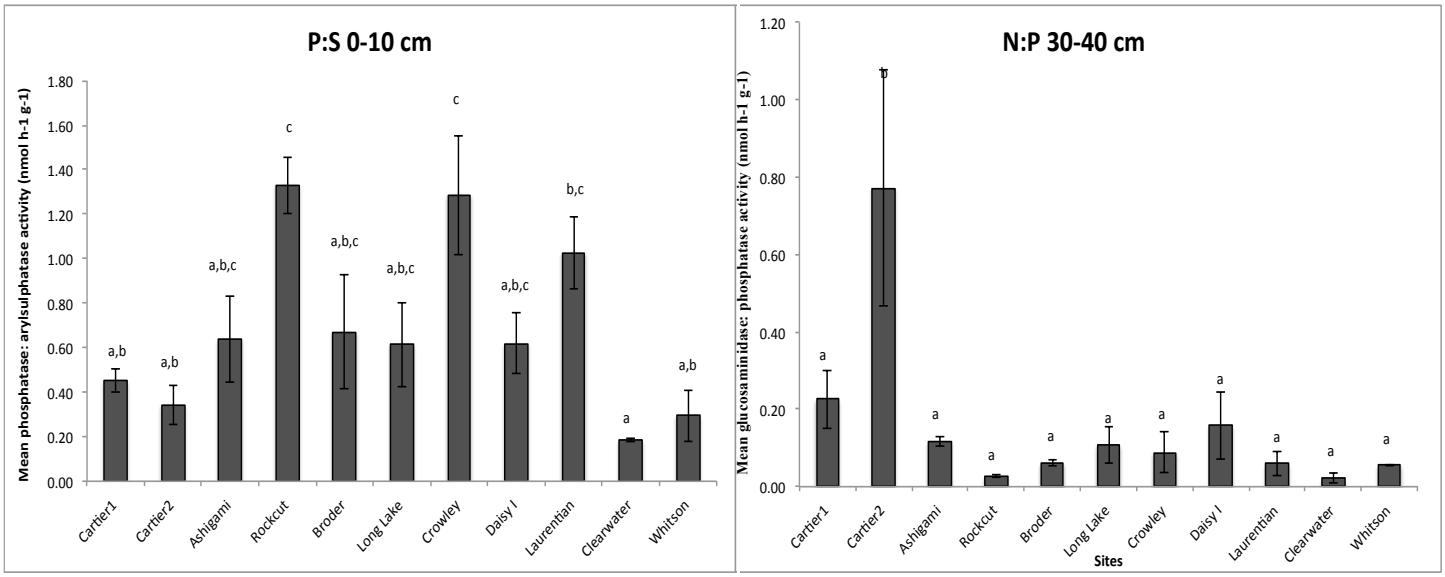



**Figure 3.33-** Mean extracellular activity of microbial communities across 11 study sites for phosphatase: arylsulphatase 0-10 cm (left), and glucosaminidase: phosphatase 30-40 cm (right), over a metal gradient. Error bars represent standard error and the letters represent the results of ANOVA tests, where shared letter indicate no significant differences between the sites.

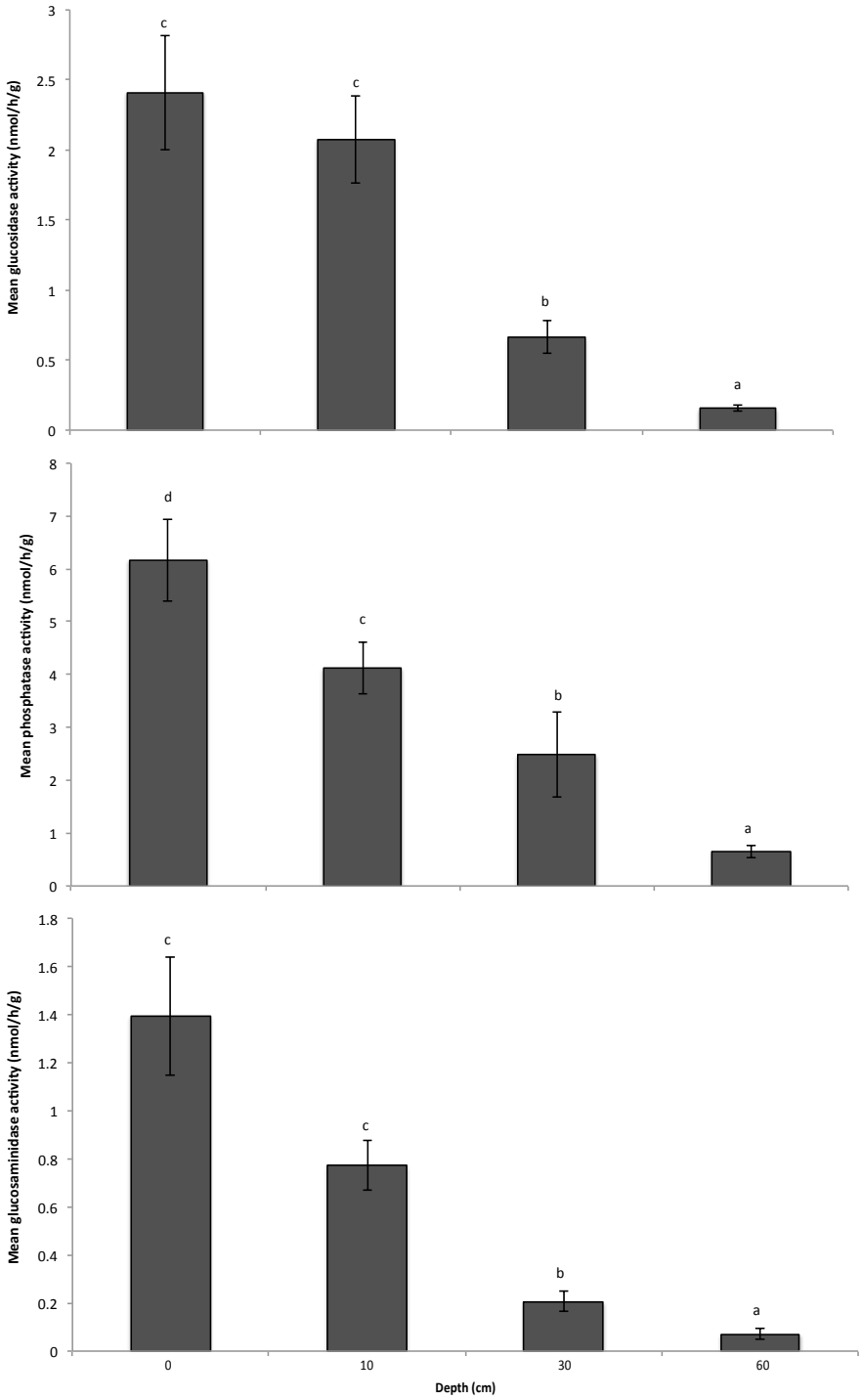



**Figure 3.34-** Mean microbial extracellular activity across 4 depths, showing the change in activity across multiple substrates (glucosidase, phosphatase and glucosaminidase respectively). Error bars represent standard error. Letters are the result of the ANOVA conducted on the log-transformed data set, and shared letters represent no significant difference between the depths.

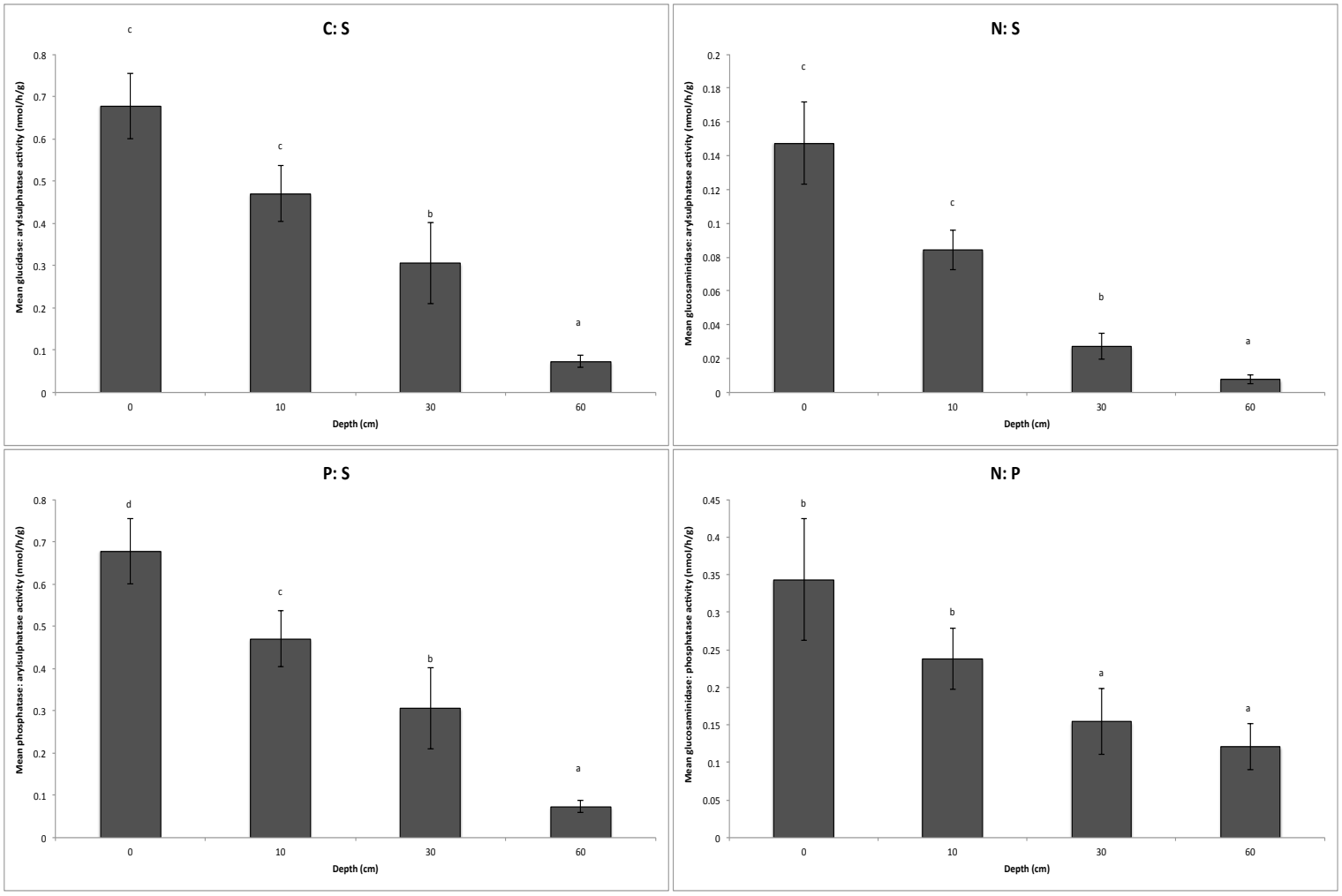



**Figure 3.35-** Mean microbial extracellular activity across 4 depths (0-10 cm, 10-20 cm, 30-40 cm and 60-70 cm respectively), showing the change in activity across multiple nutrients. Error bars represent standard error. Letters are the result of the ANOVA conducted on the log-transformed data set, and shared letters represent no significant difference between the depths.

### 3.5.2 Microbial Function: Lignases

A one-way ANOVA and post-hoc (S.N.K) tests were conducted to examine the effects of site on the microbial activity of phenol-oxidase acquiring microbes. The simple main effects analysis showed statistical significance between sites from surface to deep peat for high, intermediate high, intermediate low and low-impacted sites. There were no clear trends in phenol-oxidase activity across the sites over the different individual depths but a broad trend was observed when comparing mean phenol-oxidase activity between all three depths vertically. Firstly, phenol-oxidase activity did not decrease with depth from 10-20 cm to 60-70 cm; instead the mean activity remained the same over all three depths (Figure 3.34). Crowley (intermediate-high impacted) and Whitson (high-impacted) had phenol-oxidase activities that were statistically different at the 10-20 cm depth, but had phenol-oxidase activities that showed no statistical difference at the 30-40 cm depth or at the 60-70 cm depth (Figure 3.34). Additionally, the mean phenol-oxidase activity for Rockcut remained relatively the same at 10-20 cm and 30-40 cm depths, but almost double at the 60-70 cm depth (Figure 3.34).

A two-way ANOVA and post-hoc (S.N.K.) tests were conducted to examine the interaction for depth on the microbial activity of phenol-oxidase acquiring microbes. There was no difference in mean activity for the four depths as all four depths were roughly 6.00 μmol/h/g (Figure 3.35). There was a statistically significant difference between the two surface depths and the two deep peat depths and the 30-40 cm depth was significantly different from the 60-70 cm depth (Figure 3.35).

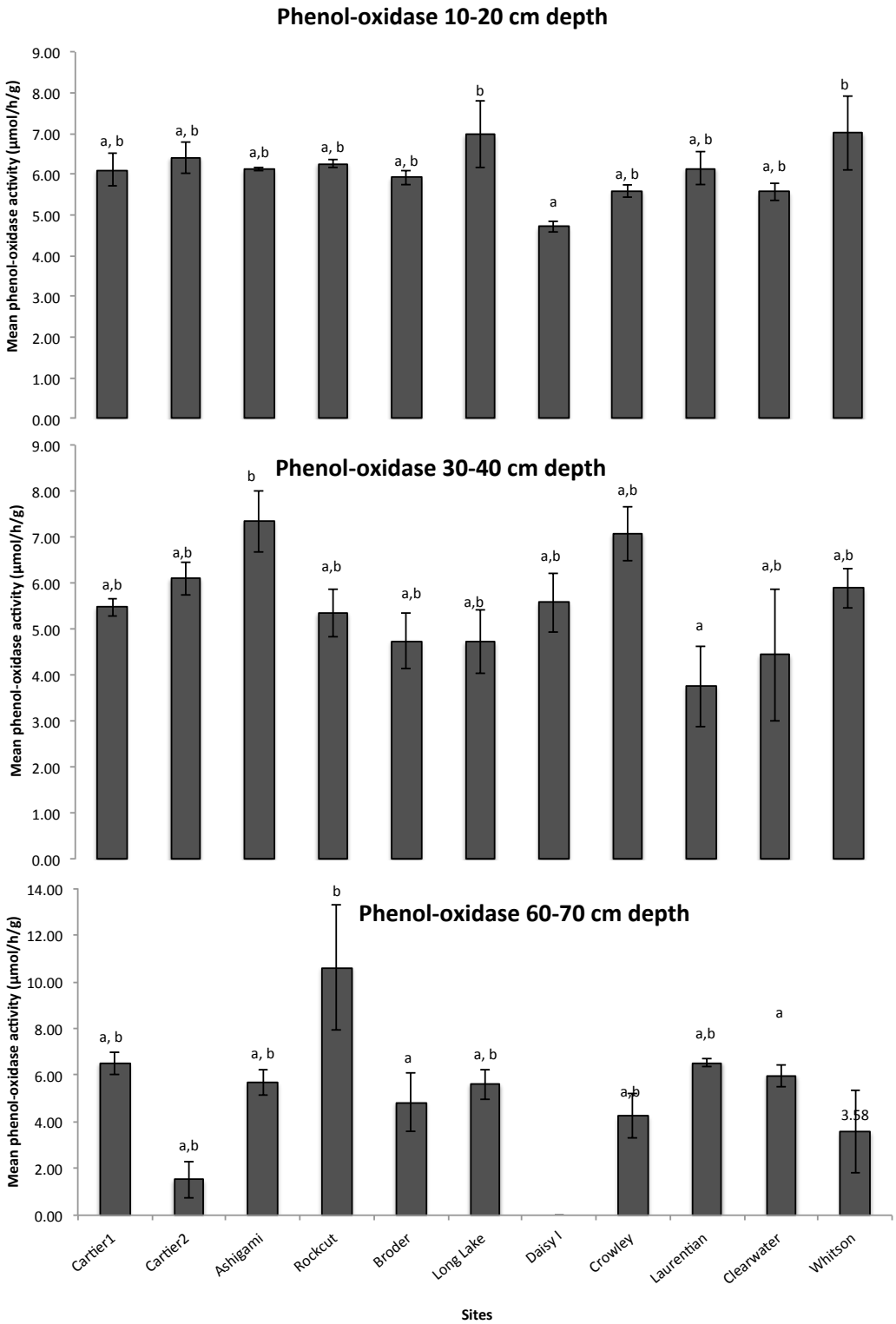



**Figure 3.36**- Mean extracellular phenol-oxidase activity of microbial communities across 11 sites at depths ranging from 10-20 cm, 30-40 cm, and 60-70 cm, respectively, over a pollution gradient. Error bars represent standard error. Letters are the result of the ANOVA and shared letters represent no significant difference between the sites.

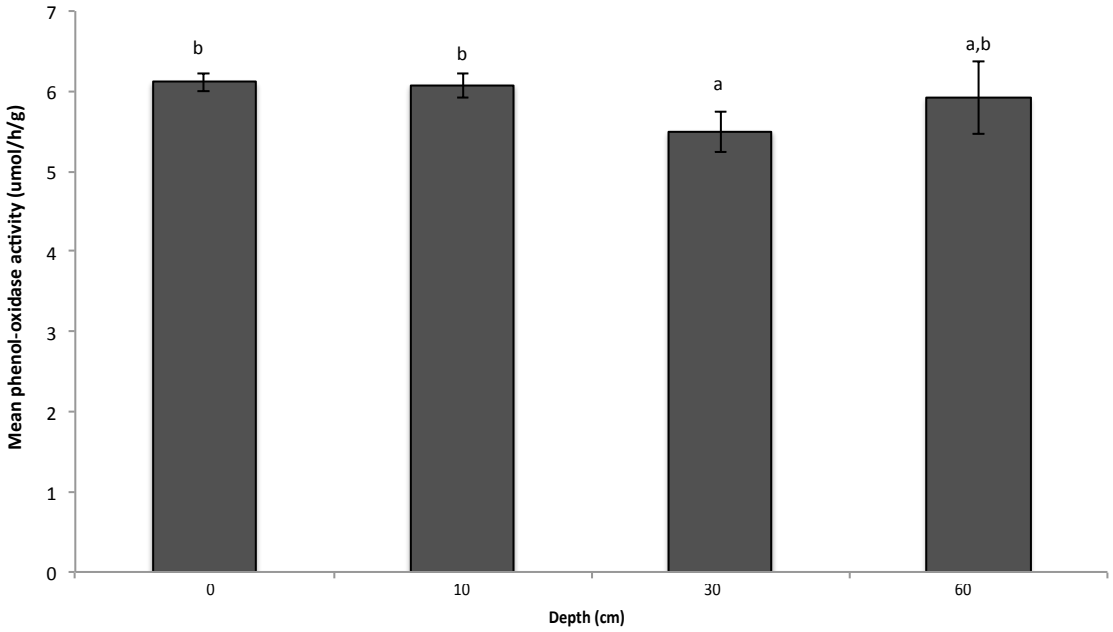



**Figure 3.37-** Mean extracellular phenol-oxidase activity of microbial communities across 4 depths. Error bars represent standard error. Letters are the result of the ANOVA conducted on the log-transformed data set, and shared letters represent no significant difference between the depths.

## 3.6 In Situ Gas Fluxes

Gas efflux analysis was conducted twice during the growing season (once at the start and then again at the end) to obtain the rate of methane ($CH_4$) and carbon dioxide ($CO_2$) production in the low-impacted sites, the intermediate-low impacted sites and the high-impacted sites. The mean rate of methane efflux was close to 0 mg/m$^2$/hr for most of the sites across the gradient and over the two time periods (Figure 3.36). However, the second collection at the end of the growing season showed an increase in variation from the mean, with Ashigami and Border (intermediate-low impacted) having the largest variability. The $CO_2$ results were contradicting over the two time periods, with the efflux rates at the start of the growing season being < 4 mg/m$^2$/hr (except for Rockcut), in the low-impacted and the intermediate-low impacted sites; and > 4 mg/m$^2$/hr in the high-impacted sites. The opposite was observed at the end the growing season with the low-impacted and the intermediate-low impacted site (except Rockcut) having higher rates of $CO_2$ efflux compared to the high-impacted sites that had low $CO_2$ efflux (< 4 mg/m$^2$/hr) (Figure 3.36).

A multivariate repeated measures analysis was conducted using Pillai's Trace and Greenhouse-Geisser for between subject and within subject effects on the methane fluxes obtained across all eight tested sites and it was concluded that there was no significant difference distances of the sites from the Copper Cliff smelter or with the time of gas collection (Table 3.14). A second multivariate repeated measures test was conducted for carbon dioxide and it was concluded that the interaction between distance from Copper Cliff and the time of collection was significant but the two variables were not significant on their own (Table 3.15).

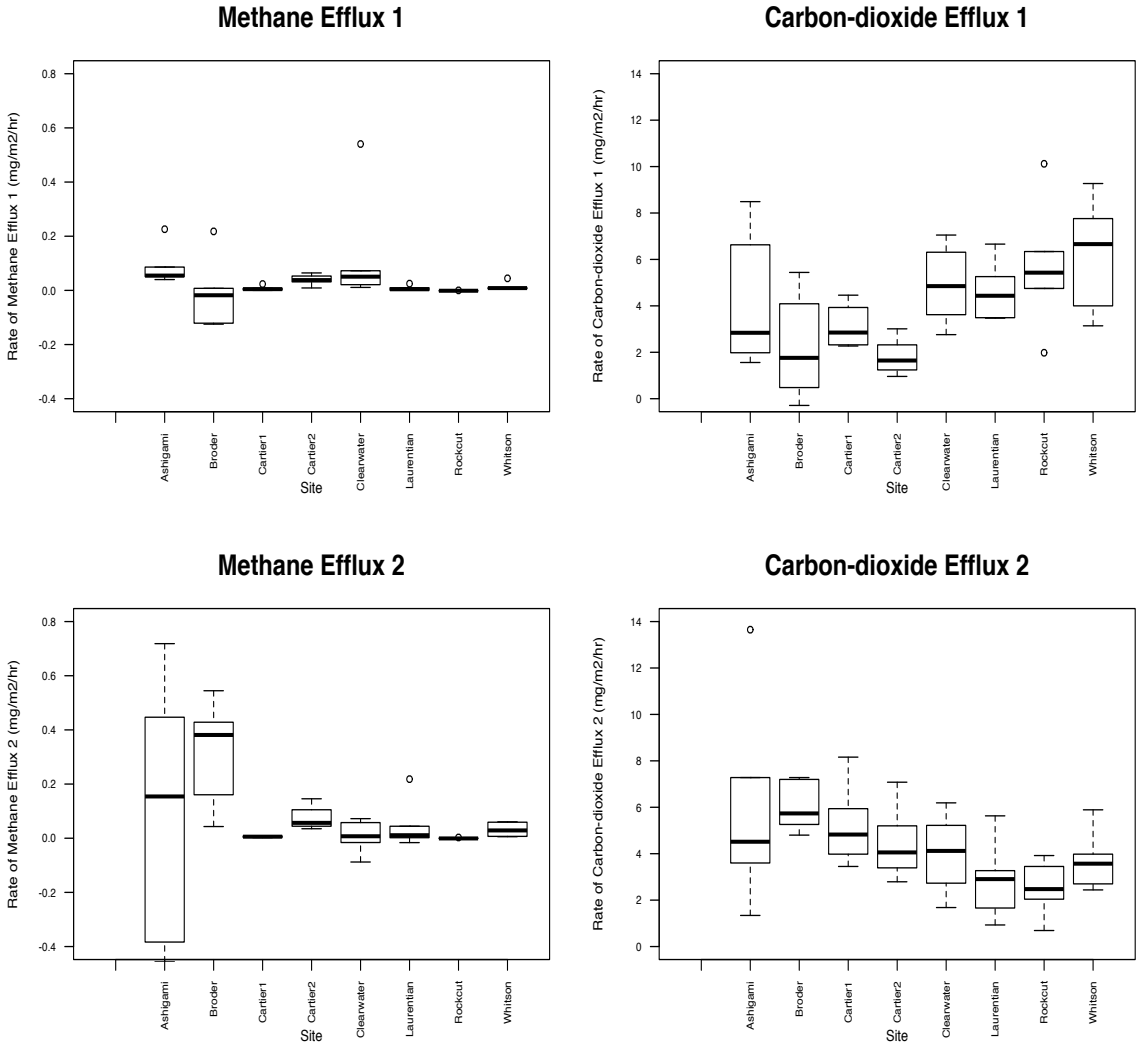



**Figure 3.38**- Mean methane and carbon dioxide efflux (mg/m$^2$/hr) across a range of metal impacted sites from low-impacted to intermediate low-impacted and high-impacted sites. Error bars represent the standard deviation of the mean.

**Table 3.14**- Multivariate repeated measures analysis was conducted using Pillai's Trace and Greenhouse-Geisser for between subject and within subject effects respectively on methane efflux over two collection times and over a distance gradient of metal.

| **Source** | **df** | **SS** | **MS** | ***F*** | ***P*** |
|---|---|---|---|---|---|
| Distance | 2 | 3.830 | 1.915 | 1.656 | 0.281 |
| Error a | 5 | 5.783 | 1.157 | | |
| Time | 1 | 1.148 | 1.148 | 5.514 | 0.066. |
| Time * Distance | 2 | 0.214 | 0.107 | 0.514 | 0.627 |
| Error b | 5 | 1.041 | 0.208 | | |
| Total | 15 | | | | |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

**Table 3.15**- Multivariate repeated measures analysis was conducted using Pillai's Trace and Greenhouse-Geisser for between subject and within subject effects respectively on carbon dioxide efflux over two collection times and over a distance gradient of metal.

| **Source** | **df** | **SS** | **MS** | ***F*** | ***P*** |
|---|---|---|---|---|---|
| Distance | 2 | 0.617 | 0.309 | 0.011 | 0.990 |
| Error a | 5 | 146.945 | 29.389 | | |
| Time | 1 | 11.036 | 11.036 | 2.971 | 0.145 |
| Time * Distance | 2 | 279.075 | 139.537 | 37.571 | 0.001*** |
| Error b | 5 | 18.570 | 3.714 | | |
| Total | 15 | | | | |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# 4 Discussion

***Impacts of the historic deposition legacy on peatlands***

Although there was considerable variability in peat and vegetation properties across the sites, there were still distinct patterns in Cu and Ni concentrations that allowed sites to be grouped from low-impacted to high-impacted. Site variability not linked to the distance from point sources may have been caused by several factors including wind direction and local topography (Keller et al., 2007), local geology and groundwater inputs (Gignac and Beckett, 1986), and plant community and interrelated peat composition linked to hydrologic and nutrient properties (Gignac and Beckett, 1986; Freedman and Hutchinson, 1980a). Copper and Ni concentrations were positively correlated with each other and negatively correlated with distance from Copper Cliff (Sudbury's largest smelting complex), indicating that as distance increased, both Cu and Ni concentrations decreased. This trend was expected because the sites within 15 km of this smelter have had the highest particulate inputs (van Alphen, 1999). This trend is also supported by an earlier study of Sudbury area wetlands by Taylor and Crowder (1983), where metal concentrations were highest within a 10 km radius of the Copper Cliff smelter. Moreover, the increase in Cu and Ni concentrations were more pronounced when looking at wetland soils (total Cu in surface soils ranging from 100 – 1300 mg/kg, and total Ni in surface soils ranging from 50-900 mg/kg (Barrett and Watmough 2016)) in the Sudbury area versus in areas outside of Sudbury such as Elliot Lake (total Cu in surface soils ranging from 2.9 – 5.3 mg/kg, and total Ni ranging from 2.6 – 8.2 mg/kg) and Killarney (total Cu in surface soils ranging from 11 – 113 mg/kg, and total Ni ranging from 20 – 115 mg/kg) (Pamer unpublished thesis, 2008). Furthermore, upland soils (mean total Cu in surface soils ranging from 2.7 – 5600 mg/kg, and mean total Ni in surface soils ranging from 7 – 3700 mg/kg (SARA, 2001)) in Sudbury were also extremely high in comparison to sites outside of Sudbury because of smelter effects.

Peat pH and humification were negatively correlated with distance from Copper Cliff, and pH was positively correlated with Cu concentration. This was important because pH can greatly affect the availability of nutrients and metals in peat, especially cations like Cu and Ni (Freedman and Hutchinson, 1980a). Souter and Watmough (2016) also reported that the peat in sites closer to the smelter was more humified. In particular, poorer fen sites such as Broder, Whitson and Laurentian that might have once been comparable to the low-impacted Cartier sites, appeared to have higher pH and metal concentrations as well as more humified peat, while richer sites like Daisy-I and Long Lake appeared to be more "natural" regarding vegetation and peat pH. The intermediate and high-impacted sites were within 15 km of Copper Cliff and therefore this deposition signature was supported by aerial deposition and proximity to the source (Freedman and Hutchinson, 1980a). I posit the following explanation of the mechanisms behind Ni, Cu, and S deposition affecting poorer sites near to the smelters: some combination of the high S and metals killed Sphagnum mosses, which are known to inhibit peat decomposition and keep pH low (van Breeman 1995), and also the extreme loading of oxidized S (although initially acidic) fuels anaerobic respiration that further contributes to peat humification and simultaneously generates alkalinity which contributes to increased pH over time (Konhauser, 2009).

Peat physical and chemical properties, and notably those that have been influenced by the relatively recent (in peat formation terms) smelting history, varied greatly over depth in the study sites. Copper and Ni concentrations were highest in the surface and subsurface depths, and lowest in the deep peat depths. In the depths below 20 cm, Cu and Ni showed little change within site and across the metal gradient, which indicated that the surface Cu and Ni concentrations in the sites are due to aerial deposition and percolation of the metals vertically appears minor, a finding also consistent with Souter and Watmough (2016). Additionally, although there was an increase in von Post humification values through depth across all study sites, where the surface depths had lower humification indices, polluted sites closer to the smelters had higher indices in the surface horizons.

***Microbial community structure and diversity along the deposition gradient***

Although microbes are a major driver of many soil processes, there is very little known regarding the relationship between atmospheric metal and associated S pollution with microbial communities. As previously stated, it was hypothesized that the microbial community structure would shift in response to the pollution legacy of the region. Principal coordinate analyses were used to observe ordination patterns in microbial communities across sites/the metal gradient and across depth, with the assumption that deeper, older peats were less impacted by more contemporary smelting activities. There was a clear separation between the microbial communities across the site and metal gradient in the Sudbury region, which indicated that each cluster of sites had distinct microbial composition. This is similar to patterns seen in other contaminated systems, such as riparian soils, sediments and acid mine drainage impacted environments (Fan et al., 2016; Bier et al., 2015; Feris et al., 2003; Kang et al., 2013; Tan et al, 2009), where heavy metals also influenced and shaped microbial community composition. There was also a distinct separation in microbial communities by depth, especially between the surface and the two deeper depth profiles, which was consistent with the presence of oxic versus anoxic conditions over depth and the organisms specialized to inhabit these zones (Andersen et al., 2013a; Lin et al., 2014a). Although the depth dependency of microbial communities in peatlands is relatively well understood, unique to my study is that the effects of pollution were muted in deeper samples, which relates both to the timescale of smelting (i.e. smelting was not occurring when deeper peat was forming), but also to the apparent lack of mobility of pollutants lower in the peat profiles.

Shannon diversity indices have been calculated and reported for many microbial communities across various ecosystems with the diversity index in an ecosystem being influenced by environmental factors such as metals and pH (Fan et al., 2016; Fierer and Jackson, 2006; Urbanova and Barta, 2016; Azarbad et al., 2015; Andersen et al., 2013a; Lin et al., 2014a). There was greater change in microbial diversity across my study sites due to the metal gradient caused

by the pollution legacy of the area (Fan et al., 2016), but also equally important, the pH of the sites (Fan et al., 2016; Fierer and Jackson, 2006; Urbanova and Barta, 2016); however as discussed above, pH measured in surface peats was at least in part a result of shifting vegetation and S reduction stimulated by pollution. A new study is currently exploring the paleoecology of some of the same sites used in my thesis, and this will confirm the role smelting has had in pH and vegetation changes over time/peat profile depth. The Shannon diversity indices in the intermediate-low and the intermediate-high sites were relatively higher than in the low and high-impacted sites. This is interesting because pH is driven by biotic factors such as the production of protons by *Sphagnum* mosses in the low-impacted sites, while high-impacted sites were observed to have lost their *Sphagnum* mosses richness, therefore their low pH is driven by abiotic factors possibly from mining. Hence, a combination of higher pH and low metal impact allows for a greater diversity of microbial species (Fierer and Jackson, 2006).

Many studies have indicated that microbial diversity decrease over depth from the surface depth (oxic) to the deep peat depth (anoxic) (Lin et al., 2014a; Andersen et al., 2013a). In this study, the microbial diversity from the shallow depths to the deep depths did not change, though the diversity was more variable with depth. The effects of metals (i.e. variability in communities in surface peat over horizontal space) were notably greater than the effects of peat depth.

***How key microbial taxa varied over the pollution gradient***

Multivariate analyses revealed a significant difference between sites per se and also between sites grouped by threshold levels of metals ($Pr(>F) < 0.05$). This indicated that both the site characteristics (e.g. fen type/trophic status, hydrologic properties, etc.) and the metal gradient affected the microbial community composition and that there was a unique microbial structure present in each site and metal impact group. Additionally, both Ca and pH ($Pr(>F) < 0.05$ and 0.001 respectively) were significantly correlated with the microbial communities. The positive correlation between pH and Ca supports the previous claims that pH is a major driver of community structure (Fan et al., 2016; Fierer and Jackson, 2006; Urbanova and Barta, 2016).

Acidobacteria and Proteobacteria were the most abundant phyla across the metal gradient making up more than half of the microbial taxa at many sites. Proteobacteria abundance has been associated with carbon availability (Fierer et al., 2007; McCaig et al., 1999), while Acidobacteria have been associated with acidic conditions in soil (Dion and Nautiyal, 2008). Acidobacteria is believed to play an important role in carbohydrate degradation under aerobic conditions (Dedysh et al., 2011). It is interesting that these two phyla are the most abundant consistently across all the sites along the metal gradient, because in other studies, like the study conducted by Urbanova and Bárta (2016), Acidobacteria abundance increased with decreasing pH and Proteobacteria decreased with decreasing pH.

The low-impacted sites had an abundance of taxa in the phyla Acidobacteria, Crenarchaeota, Euryarchaeota and Proteobacteria. Cartier1 had an abundance of Solirubrobacterales (phylum Actinobacteria), Nitrospirales (phylum Nitrospirae), and Syntrophobacterales and Rhizobiales (phylum Proteobacteria). Syntrophobacterales and Rhizobiales both have $N_2$ fixing genes and have been suggested to have nitrogen fixation potential (Lin et al., 2014b); also, Syntrophobacterales has been linked to methanogenesis under anaerobic conditions, as providers of specific fermentation products, i.e. as syntrophs of methanogens (Lin et al., 2014a; Drake et al., 2009). Caterier2 also had an abundance of Syntrophobacterales, Rhizobiales, Hyphomicrobiaceae, Methylocystaceae, and Methanomassiliicoccaceae. Hyphomicrobiaceae (phylum Proteobacteria), like Syntrophobacterales and Rhizobiales, has also been linked to N-fixation in soils (Heising and Schink, 1998; Boer et al, 2005; Kersters et al., 2006). The presence of N-fixers in these sites indicates that while most N-fixing bacteria prefer minerotrophic soils; many can still be abundant in acidic peat. Additionally, these sites had an abundance of Acidobacteria (Acidobacteriales and Ellin6513), which are responsible for the oxidation of galacturonic acids (Dedysh et al., 2011). Galacturonic acids are produced during the decomposition of Sphagnum moss (Dedysh et

al., 2011), and these low-impacted sites had the greatest abundance of mosses, therefore supporting the abundance of these microbes.

Alphaproteobacteria and Betaproteobacteria (phylum Proteobacteria), and Acidobacteriia and Solibacteres (phylum Acidobacteria) were abundant across the intermediate-low impacted sites. In a study conducted by Urbanova and Barta (2016), Alphaproteobacteria and Betaproteobacteria were positively correlated with pH, and this was also seen here in the intermediate-low impacted sites, which had relatively higher pH than the low-impacted sites. It has been suggested that Alphaproteobacteria consists of three different physiological groups: methanotrophs, chemorganotrophs, and phototrophs (Dedysh et al., 2011), and these organisms prevail in methane-emitting wetlands (Dedysh et al., 2006; Dedysh, 2009). There was an abundance of *Rhodoplanes* (phylum Alphaproteobacteria) in Ashigami and Broder, and *Bradyrhizobium* in Rockcut. *Bradyrhizobium* species belong to the family Bradyrhizobiaceae (chemoheterotrophs) that are abundant in many peat bogs (Dedysh et al., 2011). This suggests that the Alphaproteobacteria in these sites may be responsible for the oxidation of organic material, which aids with decomposition. There was also an abundance of Deltaproteobacteria in these sites, specifically, *Syntrophobacter*, *Geobacter*, and *Desulfobacca*, which are linked to increased sulfate levels in peat (Morales et al., 2006). This may indicate that Rockcut, Broder and to a lesser extent, Ashigami, are peatlands that are affected by S deposition. Although my thesis did not characterize S species concentrations in great detail, it is conceivable that the intermediate-distanced peatlands from the smelter were more impacted by S, which is transported in gaseous or semi-volatile forms, in contrast to the metals, and may have reached further.

The intermediate-high impacted sites had an abundance of Syntrophobacteraceae, Hyphomicrobiaceae, Syntrophaceae, Sinobacteraceae, and Bradyrhizobiaceae (phylum Proteobacteria), Koribacteraceae and AKIW659 (phylum Acidobacteria), Dehalococcoidaceae (phylum Chloroflexi), and Methanomassiliicoccaceae (phylum Euryarchaeota). The abundance of Chloroflexi in the intermediate-high impacted sites is supported by other studies that have also

found Chloroflexi in other metal-impacted sites (Azarbad et al., 2015, Chodak et al., 2013). The abundance of these organisms in metal-impacted sites indicates that they may be adapted to extreme environments (Azarbad et al., 2015) like Cu and Ni impacted environments, but also that these organisms could possibly play an important role in metal impacted soils. Additionally, Methanomassiliicoccaceae, like other methanogenic bacteria belonging to the class Thermoplasmata, is described to have the ability to combine methylotrophy with methanogeny by utilizing methylamines with the formation of $CH_4$ (Poulsen et al., 2013). The abundance of these methanogens in the more impacted sites indicates that these $CH_4$ producers are metal tolerant.

In the high-impacted sites, there was an abundance of Hyphomicrobiaceae, Methylocystaceae, Syntrophaceae and Syntrophobacteraceae (phylum Proteobacteria), Thermodesulfovibrionaceae (phylum Nitrospirae), Gaiellaceae (phylum Actinobacteria), and Methanomassiliicoccaceae (phylum Euryarchaeota). As previously mentioned, Syntrophobacteraceae and Hyphomicrobiaceae are linked to $N_2$ fixation in soils (Heising and Schink, 1998; Boer et al, 2005; Kersters et al., 2006) and also to mutualistic plant symbiotic relationships (Denison and Kiers, 2011). Additionally, Thermodesulfovibrionaceae are a group of anaerobic sulfate reducing bacteria, capable of using hydrogen as an electron donor and also capable of fermenting pyruvate and other organic material (Haouari et al., 2008). The presence of Syntrophaceae, Syntrophobacteraceae and Thermodesulfovibrionaceae indicates that there are increased levels of sulphate in these sites, as these microbes are linked to high sulfate concentrations (Morales et al., 2006; Haouari et al., 2008). These sites are close to the Copper Cliff smelter and they all have high metal concentrations, and have also been exposed to some of the highest local atmospheric SO4 on earth (e.g. in early smelting practice “roast beds”) before taller stacks and S capturing technologies were implemented. Moreover, there was an abundance of Methanomassiliicoccaceae (methanogenic) and Methylocystaceae (methanotrophic) in these sites, which indicates a strong dynamic in $CH_4$ production and consumption. Methylocystaceae

are dominated and very active microbes in northern wetlands, which in this case, they are dominant even in these heavy metal-impacted sites.

***How key microbial groups varied through depth***

A number of studies have looked at the importance of the vertical gradient in wetland and peatland soils and how this influences microbial community diversity and composition (Lin et al., 2014a; Andersen et al., 2013a). Here, although there were no significant differences in microbial community structure by depth universally observed across all sites (noting variable site type, vegetation and particularly water table depths across sites), there were significant differences in the communities when looking at depth within a given site. Based on the community analyses, this difference in depth communities appear to be linked to differences in oxygen or alternative electron acceptor availability with depth. In the PCA, it was revealed that the surface depth was correlated with metals such as Cu, Ni and Al, while the two deep depths were correlated with moisture, von Post, pH and temperature. This perhaps supports that smelting impacts were seen principally in the surface, most recently formed peat. That metals do not impact the entire peat deposit, might mean that with site reclamation and re-establishment of natural Sphagnum moss dominated communities at the surface, these sites could be restored, at least regarding their microbial biogeochemical functioning.

The acrotelm sample (10-20 cm) in this study was largely oxic; and also the horizon with most (recent) influence of carbon input from plant production at the surface (Lin et al., 2014a). This layer had an abundance of Acidobacteriales and Ellin6513 (phylum Acidobacteria), Rhizobiales (class Alphaproteobacteria). Notably Acidobacteria and Alphaproteobacteria have been reported to have the ability to decompose carbohydrates in acrotelm peats (Dedysh et al., 2011; Lin et al., 2014a). These microorganisms are capable for utilizing a range of plant-derived polymers, including most sugars, some heteropolysaccharides, and several low molecular weight carbon substrates, under acidic conditions (Dedysh et al., 2011). The mesotelm (zone between oxic and anoxic layers, typically falling near or at my 30-40 cm samples) had an abundance of

Bacteroidales (phylum Bacteroidetes), Rhizobiales and Syntrophobacterales (phylum Proteobacteria), Acidobacteriales and Ellin6513 (phylum Acidobacteria), and Nitrospirales (phylum Nitrospirae). Organisms from the phylum Acidobacteria are likely key in the decomposition of carbohydrates (similar to the acrotelm) under aerobic conditions (Lin et al., 2014a). Additionally, both Rhizobiales and Syntrophobacterales have $N_2$ fixation potential (Lin et al., 2014b), indicating their potential role as N-fixers in the mesotelm; and Syntrophobacterales support methanogenesis under anaerobic conditions as mentioned above (Lin et al., 2014a). Bacteroidetes is a bacteria phylum that has Gram-negative species with aerobic or anaerobic traits; these species have a wide range of physiological diversity (Bauer et al., 2006; Dhal et al., 2011), making them capable in thriving in the mesotelm. Lastly, in the catotelm (deep anoxic layer, 60-70 cm), there was an abundance of Rhizobiales, Syntrophobacterales, Acidobacteriales, Ellin6513, and Solirubrobacterales (phylum Actinobacteria). This layer had a high degree of humification, as previously stated, and it has a bulk store of stable, recalcitrant carbon, termed the "carbon bank" (Clymo, 1984). The role of Acidobacteria is limited at this depth (Dedysh, 2011), but Proteobacteria such as Syntrophobacterales support methanogenesis under anaerobic conditions (Lin et al., 2014a). Members of the kingdom Archaea are more abundant in this layer (Lin et al., 2014a) and Thermoplasmata (phylum Euryarchaeota) and NRP-J (phylum Crenarchaeota) were present at depth in many sites. Thermoplasmata, like most methanogens, thrive in the deep anoxic peat soils through anaerobic respiration, but many studies have suggested that their activity is slow due to low substrate availability (Urbanova and Barta, 2016; Yrjala et al., 2011).

***How environmental factors across the pollution gradient influenced key organisms and indicator species***

The microbial indicator "species" within the > 1% abundant communities showed that there were a number of organisms that effectively represented the variability across sites, depths, and the metal gradient. In other words, these taxa play a key role in defining the differences

between microbial communities. Nine unique OTUs were determined to be "indicator species", with Acidobacteriaceae (oxidizing simple and complex carbohydrates), *Bradyrhizobium* and Syntrophaceae (potential N-fixing ability), and Ellin515 being potential indicators across the sites. Ellin515 (phylum Verrucomicrobia) is generally negatively correlated with copper contamination in soils, as seen in the study by Berg et al., (2012); therefore this species acting as a potential indicator across site is very interesting. However, the physiological role of Ellin515 in natural environments is not yet known (Rastogi et al., 2010). Over the three depths, the potential indicator species included Syntrophobacteraceae (potential N-fixing ability) and Koribacteraceae. Lastly, across the metal gradient, Koribacteraceae and TM1 (oxidizing simple and complex carbohydrates), and Phycispaerae (phylum Planctomycetes) represented the potential indicator species. TM1 have been identified in grassland soils (Cho et al., 2008), and Planctomycetes (Phycispaerae) are an abundant bacterial group found in Sphagnum dominated wetlands (Dedysh et al., 2006). Planctomycetes are capable of degrading heteropolysaccharides but not cellulose or chitin (Dedysh et al., 2011). These species are linked to depth in other studies and grow best in aerobic conditions (Dedysh et al., 2011).

Univariate analyses on key microbial taxa and indicator species against environmental factors revealed that many taxa were correlated metals (Cu and Ni), minerals (Ca) and pH. JH-WHS47 (phylum NC10, OTU14), SBla14 (phylum Proteobacteria, OTU12), Ellin515 (phylum Verrucomicrobia, OTU19), and *Syntrophus* (phylum Proteobacteria, OTU35) had a significant relationship with Ca, Al, degree of humification and pH. This indicated that these organisms were affected by peat mineral content and physical properties. Ellin6513 (phylum Acidobacteria, OTU6) had a significant relationship with Ca, temperature and pH, while Methylocystaceae (phylum Proteobacteria, OTU10) had a significant relationship with Cu, Ni, Ca, Al, and pH. Other OTUs that had a significant relationship with metals included TM1 (phylum Acidobacteria, OTU1), which was significantly related to Cu, Ni, K, temperature and pH; and Syntrophobacteraceae (phylum Proteobacteria, OTU15), which was significantly related to Cu,

Ni, Al and pH. These metal impacted microbes were significantly related to Cu, Al and Ni indicating that they have a tolerance or affinity to metals in peat. Lastly, *Candidatus Koribacter* (phylum Acidobacteria, OTU34) had a significant relationship with Cu, Ni, and temperature, while BSV26 (phylum Chlorobi, OTU76) had a significant relationship with Ni, von Post and pH. These organisms were also significantly related to environmental factors such as temperature, which is controlled by seasonality and climate; also with peat humification; and with pH, which as noted above regarding my work (and as seen in many other studies), was one of the major drivers of community structure and diversity.

***How plants appeared to indirectly mediate pollution effects on microbial communities***

Along the metal gradient, *Sphagnum* moss richness was sparse or absent in many of the high and intermediate-high impacted sites, but the intermediate- low and low-impacted sites all had *Sphagnum* mosses present, with the low-impacted sites having the highest richness. Similar *Sphagnum* mosses richness was observed in other studies of the area, indicating a lack of tolerance of mosses to metal pollution (Barrett and Watmough, 2015; Gignac and Becket, 1986). *Sphagnum* mosses richness was significantly correlated with distance from the Copper Cliff smelter, where richness increased away from the smelter. Sites such as Daisy-I, Broder, Crowley, Laurentian and Clearwater were correlated with Cu and Ni, but also with humification and shrub cover such as with *Camaedaphne calyculata*. As previously mentioned, *Sphagnum* mosses makes up to 90% of many peats due to its recalcitrant and antibacterial properties, enabling it to inhibit decomposition compared to vascular plant litters (Andrus, 1986). These non-vascular plants also have the ability to maintain a high watertable, which alters hydrology and pore water biogeochemistry (Andrus, 1986). Beyond eliminating its antimicrobial properties, sparseness or absence of *Sphagnum* mosses richness in sites close to the smelters may also have caused watertable fluctuations, creating favorable conditions for decomposition (Freeman et al., 1997). Watertable fluctuations, along with less recalcitrant vascular plant litter may have resulted in higher rates of decomposition; hence more humified peat (Souter and Watmough, 2016).

***Microbial activities along the pollution gradient and through peat depth and potential linkages between diversity and activity***

It was hypothesized that microbial activity would decrease closer to the smelter due to metal pollution having a possibly negative effect on the functionality of the microbial community. It was also hypothesized that microbial community function would be greater at the surface because the surface depth should have more labile substrates and potentially greater microbial diversity. In general, phosphatase had the highest activity of the four-hydrolase enzymes, which indicated that available P might be limited in the peatlands in the Sudbury area. This P limitation was pronounced in the intermediate-sites at the 0-10 cm depth, where these sites had the highest phosphatase activity, indicating that P is limiting in their environments (i.e. because microbes are putting metabolic resources into acquiring P). This trend was also seen in the P:S activity across the sites at the 0-10 cm depth, where Crowley and Rockcut had the highest P:S ratio and this indicated that these sites potentially had limiting P but higher S in the sites due to the impact of the smelter. *Candidatus Solibacter* (phylum Acidobacteria) has been suggested to play a dominant role in phosphatase activity (Lin et al., 2014b), and their abundance in the intermediate sites may support that. Lin et al. (2014a; 2014b) determined that P availability strongly influences microbial growth and also the decomposition of organic material (Lin et al., 2014b; Lin et al., 2014a). Phosphorus limitation affects microbial processes such as $N_2$ fixation, since N fixers require P to function (Vitousek and Hobbie, 2000; Reed et al., 2013; Vitousek et al., 2013). Nitrogen fixers include Rhizobiales and Syntrophobacterales (Lin et al., 2014b), which were abundant across the sites but Rhizobiales were more abundant in the intermediate sites. The overall glucosidase activity was generally high, indicating the importance of carbon (C) in these sites. The highest glucosidase activity occurred in the high-impacted sites, indicating that these sites potentially have low C availability in their peatland environments. If this is true, then it explained why these sites took on the energy expensive route that enzyme activity brings. Acidobacteria has been suggested to play an important role in carbohydrate

degradation (Lin et al., 2014a), and these organisms are the most abundant in the high-impacted sites and maybe responsible for glucosidase activities. Glucosaminidase at the 30-40 cm depth had the lowest enzyme activities overall, and the low and intermediate-low impacted sites generally had the highest glucosaminidase activities (also Daisy and Whitson), indicating that nitrogen (N) availability was low in these sites. Additionally, N:P activity ratios were also highest in the low-impacted sites, which also indicated low N and availability in these peatlands. Lastly arylsulphatase activity was generally the same across all the sites, with Daisy-I having the highest arylsulphatase activity and Long Lake having the lowest. Daisy-I and Long Lake were in close proximity and similar distance from the smelter in the same direction and so they should have had the same abundance of sulphur (S), but Daisy-I was limed in the past, which may have decreased the concentration of available S.

Vertically, the highest enzyme activities for glucosidase, glucosaminidase, phosphatase, and C:S, N:S, P:S, N:P activity ratios occurred in the acrotelm, and the lowest activities occurred in the catotelm. This could be because of the change from oxic (acrotelm) to anoxic (catotelm), where microbial activity is highest in the presence of oxygen. There were orders of magnitudes of difference between the enzyme activity at the surface depths (0-10 cm and 10-20 cm) and the two deep depths (30-40 cm and 60-70 cm), and each depth was significantly different from the other. Freeman et al. (2001 and 2004) suggested that an increase in oxygenation of soil could alleviate the inhibition of phenolic compounds, which could activate hydrolase enzymes activities. The acrotelm is the most oxygenated peat depth and this could explain the high hydrolase activities at the surface depth. Secondly, *Sphagnum* mosses account for a relatively large portion of peat organic matter and these mosses are made up of aromatic compounds, which are recalcitrant to microbial decomposition (Lin et al., 2014b). Aromatic compounds increase with depth, resulting in less decomposition of peat matter with depth (Lin et al., 2014b). Proteases also decrease with depth, which supports my findings that N acquiring activity is highest at the surface depth (Lin et al., 2014b).

Lignase assays (per-oxidase and phenol-oxidase activities) revealed some of the highest enzyme activities across the sites and over the depths for these enzymes. Nevertheless, there were no consistent patterns in phenol-oxidase across the pollution gradient or vertically, and it appeared that phenol-oxidase activity was the same across site and depth (although there were some significant differences by site and depth). It was unclear why there were no changes in lignases across the study sites, but lignases (phenol-oxidase especially) have been linked to Proteobacteria and Acidobacteria in peatlands (Lin et al., 2014b), and these phyla were exceptionally abundant across all sites. Proteobacteria and Acidobacteria may have played an integral role in generating phenol-oxidases, and therefore, with relatively equal abundance of these organisms across the sites and depths and the addition of equal substrate, these organisms potentially behaved in the same manner by utilizing the substrate and producing phenol-oxidases at the same rate.

***In situ methane and carbon dioxide fluxes***

Methane ($CH_4$) and ($CO_2$) fluxes in peatlands over a metals contamination gradient (subset of 8 sites) was examined to order to determine the effect of smelting on gas efflux to the atmosphere. There was no significant difference in $CH_4$ concentrations across the sites or the gas collection times, but the $CH_4$ concentrations were nevertheless considered relatively significant because of the fairly low *p*-value ($p = 0.066$) and the limited measurement campaign undertaken in the field. Methane production and consumption by microbes were relatively the same in August, when watertable was lower and temperature was higher, but at the end of the growing season watertable levels increased across the sites and temperature decreased, which allowed for methane production and efflux to increase (Bubier et al., 1993; Pelletier et al., 2007). While $CH_4$ concentrations were low throughout the field season, the low-impacted and the intermediate-low impacted sites had relatively higher $CH_4$ efflux than the high-impacted sites (except Cartier1 and Rockcut), which indicates that higher Cu and Ni concentrations potentially limited $CH_4$ fluxes in the high-impacted sites. Nickel is a trace metal that enhances the production of $CH_4$ by

methanogens in peatlands (Basiliko and Yavitt, 2001), and the high-impacted sites had the highest concentrations of Ni, potentially causing greater $CH_4$ production. In contrast, Cu is known to play a significant role in regulating methane oxidation in microbes and potentially leading to greater $CH_4$ consumption (Chidambarampadmavathy et al., 2015), and the high-impacted sites had the highest Cu concentrations. This contrasting effect, along with the confounding effect of poorer carbon quality (non-*Sphagnum* litter) may explain the low methane emission in these high-impacted sites. Additionally, Szkokan-Emilson et al., (2014) reported that S concentration was much higher in the sites closer to the super-stack. Peatland $CH_4$ concentrations can be suppressed by increased sulphate deposition (Gauci et al., 2004), which supports my findings where the high-impacted sites had lower $CH_4$ concentrations compared to the reference sites. Higher sulphate concentrations can potentially lead to reduced $CH_4$ concentrations because of the ability of sulphate as an electron acceptor, to channel electrons to sulphate reducing bacteria (more thermodynamically efficient) over methanogenic bacteria, hence limiting $CH_4$ production. In general, pH can also affect methanogenic and methanotrophic activities in peatlands as most methanogens favor a higher pH (Garcia et al., 2000), while methanotrophs favor more acidic conditions (Kamal and Varma, 2008). Therefore, the overall low $CH_4$ concentrations are potentially caused by the lack of abundance of methanogens and the greater abundance of methanotrophs. Lastly, it should be noted that $CH_4$ concentration was consistently higher in Cartier2 versus Cartier1, but in a study by Yavitt et al. (1997), forested peatlands (like Cartier1) had slower $CH_4$ production. This was because of very poor peat quality from woody plant (Yavitt et al., 1997).

The efflux of $CO_2$ was variable across sites and over the two gas collection times in June and at the end of the growing season. There was a significant relationship between $CO_2$ efflux with distance and gas collection time, with $p = 0.001$. Peat respiration depends on temperature, moisture content, the nature of the peat material present, plant activities, and the microbial communities in the sites (Chapman and Thurlow, 1998), but the cause of the fluctuations was

unclear since the general moisture and temperature trends were the same across sites. Further studies would have to be done to determine how net $CO_2$ efflux is influenced by peat organic matter quality and microbial community structure.

***Studies of peatland ecology rarely report the effects of mining/smelting on microbial communities and microbial function, especially in terms of Cu and Ni effect. My primary objectives were to examine the ways in which the smelting legacy in Sudbury impacted the microbial community structure and phylogenetic diversity in peatlands, to examine how the metal deposition legacy of the area affected extracellular enzymatic reactions and the net emissions of $CH_4$ and $CO_2$, and to determine how microbial community structure links to biochemical/biogeochemical functioning. I found that the smelting legacy and the biochemical and biogeochemical functioning of the region affected the microbial communities by selecting for distinct communities according to the Cu and Ni concentrations, along with pH, mineral concentrations (especially Ca), substrate quality and humification; and that the diversity was affected by metal impact more so than depth impact, with the highest microbial diversity in the sites with higher pH and lower metal concentrations. I also found that metal concentrations in the sites and depths affected microbial enzymatic reactions, with depth having the greater reaction over sites. The surface depths had orders of magnitudes higher reactions for all enzymes over the deep depths, which can be explained by greater microbial community function at the surface because the surface depths had more labile substrates and potentially greater microbial diversity. Additionally, $CH_4$ was lowest in the sites closer to the smelter indicating that higher S, Ni and Cu from the super-stack, and pH to some extent, along with poor substrate quality potentially affected microbially mediated $CH_4$ efflux.***

This study is one of the first of its kind, and it lays the foundation for future studies to better understand the confounding effects of metal impact on microbial communities. All the peatland sites studied had some level of metal impact because of the proximity to the Copper Cliff smelter, and therefore to completely remove the Cu and Ni signature, further study sites that

are farther away from the smelters is required. Without sites outside of the Sudbury area, it is difficult to separate the effects of metal impact from plant, nutrient, or erosion impact. It was also be interesting to observe the effects of smelting on peatland bogs. Additionally, it would be beneficial to have the ability to classify the microbes in this study to genus or species level, which would aid in determining specific characteristics/relationships between site characteristics and microbial species. Lastly, having a more thorough in situ greenhouse gas emissions study would aid in determining patterns between site biogeochemistry, gas flux and potentially the microbial species responsible.

# 5 General Conclusion

The sites showed a difference in the metal gradient with microbial diversity, community structure, and microbially mediated gas efflux differing between areas closest to current and historical smelters to areas 55-km away. Metal impact and pH were the two major drivers of microbial diversity and community with pH possibly driving metal availability. This is seen where the sites with the lowest pH having the lowest microbial diversity, and sites with the highest pH having the highest microbial diversity; and all sites had very distinct communities. There was a significant difference within each peatland where vertical peat profiles showed significant difference in microbial enzyme function for the four depths, with the surface depth having the highest enzyme activity. I deduced that microbial function differed over depth because of the difference between the aerobic and the anaerobic communities, where the aerobic communities appear to be more active. This high enzymatic reactions at the surface depths indicated that there was a higher presence of labile nutrients present across the sites. The sites were limited in carbon, phosphorus and nitrogen, and therefore they all had significant enzymatic reactions to the addition of these substrates, while the enzymatic reaction to sulphur was unchanged through the sites indicating that the potential abundance of sulphur from aerial acid deposition alleviated any sulphur limitations that may be present. Phosphorus activities was highest in the intermediate sites and these sites had an abundance of *Candidatus Solibacter*, which has been suggested to play a dominant role in phosphatase activity. Acidobacteria has been suggested to play an important role in carbohydrate degradation, and these organisms were the most abundant in the high-impacted sites and maybe responsible for high glucosidase activities in these sites. Lastly, methane efflux was higher in impacted sites because of the increased concentrations of Nickel, Copper, pH and possibly Sulphur restricting microbially mediated gas efflux through the inhibition of methane production. Additionally, the low substrate quality in these sites, caused by the loss of Sphagnum could also affect the rate of methane efflux.

# 6 Reference


Abedin, J., Beckett, P., & Spiers, G. (2012). An evaluation of extractants for assessment of metal phytoavailability to guide reclamation practices in acidic soilscapes in northern regions. *Canadian Journal of Soil Science*, *92*(1), 253-268.

Adamo, P. S., Dudka, M.J. Wilson and W.J. McHardy. (1996). Chemical and Mineralogical Forms of Cu and Ni in Contaminated soils from the Sudbury Mining and Smelting region, *Canada. Environmental Pollution*, Vol. 91, No. 1, pp. 11-19.

Adamo, P., Dudka, S., Wilson, M.J., McHardy, W.J. (2002). Distribution of trace elements in soils from the Sudbury smelting area (Ontario, Canada). Water Air Soil Pollut. 137, 95-116.

Amha, Y., Bohne, H., & Alsanius, B. (2015). Patterns of Fungal and Bacterial Carbon Mineralization Across Northern European Peatlands. *Geomicrobiology Journal*, *32*(10), 914-923.

Amiro, B.D., and Courtin, G.M. (1981). Patterns of vegetation in the vicinity of an industrially disturbed ecosystem, Sudbury, Ontario. *Can. J. Bot.* **59**: 1623 – 1639.

Amon, J. P., Thompson, C.A., Carpenter, Q.J. and J. Miner. (2002). Temperate Zone Fens of the Glaciated Midwestern USA. *Wetlands*. 22: 2. Pg. 301-317.

Andersen, R., Chapman, S.J., and Artz, R.R.E. (2013a). "Microbial Communities in Natural and Disturbed Peatlands: A Review." *Soil Biology and Biochemistry* 57: 979–94. doi:10.1016/j.soilbio.2012.10.003.

Andersen, R., Pouliot R., and Rochefort, L. (2013b). "Above-Ground Net Primary Production from Vascular Plants Shifts the Balance Towards Organic Matter Accumulation in Restored Sphagnum Bogs." *Wetlands* 33, no. 5: 811–21. doi:10.1007/s13157013-0438-5.

Andrus, R. (1986). Some aspects of Sphagnum ecology. Can. J. Bot. 64 (2), 416–426.

Azarbad, H., Nikli ska, M., Laskowski, R., van Straalen, N. M., van Gestel, C. A. M., Zhou, J., He, Z., Wen, C., and Roling, W. F. M. (2015). "Microbial Community Composition and Functions Are Resilient to Metal Pollution along Two Forest Soil Gradients." *FEMS Microbiology Ecology* 91, no. 1: 1–11. doi:10.1093/femsec/fiu003.

Barlett, K.B., and Harris, R.C. (1993). Review and assessment of methane emissions from wetlands. *Chemosphere* 26, 261–320.

Barrett, S., and Watmough, S. (2016). "Factors Controlling Peat Chemistry and Vegetation Composition in Sudbury Peatlands after 30 Years of Pollution Emission Reductions." *Environmental Pollution* 206: 122–32. doi:10.1016/j.envpol.2015.06.021.

Basiliko, N. and Yavitt, J. B. (2001). Influence of Ni, Co, Fe, and Na additions on methane production in Sphagnum dominated Northern American peatlands. Biogeochemistry. 52(2): 133–153.

Basiliko, N., Knowles, R. and Moore, T. R. (2004). Roles of Moss Species and Habitat in Methane Consumption Potential in a Northern Peatland. *Wetlands*. 24:1. Pg. 178-185.

Bauer, M., Kube, M., Teeling, H., Richter, M., Lombardot, T., Allers, E., Würdemann, C.A., Quast, C., Kuhl, H., Knaust, F. (2006). Whole genome analysis of the marine Bacteroidetes 'Gramella forsetii' reveals adaptations to degradation of polymeric organic matter. Environ. Microbiol. 8 (12), 2201e2213.

Bedford, B. L., Walbridge, M. R., and Aldous, A. (1999). Patterns in nutrient availability and plant diversity of temperate North American wet- lands. *Ecology* 80, 2151–2169.

Begak, D.A. (1926). Microbiological study of the high-moor peatland: quantitative assessment of bacteria in the high-moor peat. Pochvovedenie 2, 64e75.

Berg, J., Brandt, K.K., Abu Al-Soud, W. (2012). Selection for Cu-tolerant bacterial communities with altered composition, but unaltered richness, via long-term Cu exposure. Appl Environ Microb 78:7438–46.

Bier, R.L., Voss, K.A., Bernhardt, E.S. (2015). Bacterial community responses to a gradient of alkaline mountaintop mine drainage in Central Appalachian streams. ISME J. 9 (6), 1378e1390.

Binet, S., Gogo, S., Laggoun-Defarge, F. (2013). A water-table dependent reservoir model to investigate the effect of drought and vascular plant invasion on peatland hydrology. Journal of Hydrology 499, 132-139. http://dx.doi.org/10.1016/j.jhydrol.2013.06.035

Boer, W.D., Folman, L.B., Summerbell, R.C. & Boddy, L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 29: 795–811.

Bragazza, L., Buttler, A., Habermacher, J., Brancaleoni, L., Gerdol, R., Fritze, H., Hanajik, P., Laiho, R., and Johnson, D. (2012). High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. Global Change Biology 18, 1163–1172, doi: 10.1111/j.1365-2486.2011.02585.

Brown, S.M., Campbell, L.T., Lodge, J.K. (2007). Cryptococcus neoformans, a fungus under stress. Curr Opin Microbiol 10(4):320–325

Bubier, J. L., Costello, A., Moore, T. R., Roulet, N. T. and Savage, K. (1993). Microtopography and methane flux in boreal peatlands, northern Ontario, Canada. Can. J. Bot. 71: 1 056–1 063.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. (2010). Nature Methods, doi:10.1038/nmeth.f.30

Chapman, S.J., Thurlow, M. (1998). Peat respiration at low temperatures. Soil Biol. Biochem. 30, 1013–1021

Chidambarampadmavathy, K., Obulisamy, P., & Heimann, K. (2015). Role of copper and iron in methane oxidation and bacterial biopolymer accumulation. *Engineering in Life Sciences*, *15*(4), 387-399.

Chirino, C., Campeau, S., and Rochefort, L. (2006). *Sphagnum* establishment on bare peat: The importance of climatic variability and *Sphagnum* species richness. *Appl. Veg. Sci.*9: 285-294.

Cho, S.-T., Tsai, S.-H., Ravindran, A., Selvam, A., and Yang, S.-S. (2008). "Seasonal Variation of Microbial Populations and Biomass in Tatachia Grassland Soils of Taiwan." *Environmental Geochemistry and Health* 30, no. 3: 255–72. doi:10.1007/s10653-007-9113-1.

Chodak, M., Golebiewski, M., Morawska-Ploskonka, J. (2013). Diversity of microorganisms from forest soils differently polluted with heavy metals. Appl Soil Ecol; 64:7–14.

Clymo, R.S. (1965). Experiments on the breakdown of *Sphagnum* in two bogs. *J. Ecol.* 53, 747–758.

Clymo, R.S. (1984). The limits to peat bog growth. Philos. Trans. R. Soc. B 303:605–654. http://dx.doi.org/10.1098/rstb.1984.0002.

Clymo, R., Bryant, C. (2008). Diffusion and mass flow of dissolved carbon dioxide, methane, and dissolved organic carbon in a 7-m deep raised peat bog. Geochimica et Cosmochimica Acta 72, 2048e2066.

Cole, K.L., Engstrom, D.R., Futyma, R.P., Stottlemyer, R. (1990). Past atmospheric deposition of metals in northern Indiana measured in a peat core from Cowles Bog. Environ. Sci. Technol. 24, 543-549.

Conrad, R. (1999). Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. FEMS Microbiol. Ecol. 28(3): 193–202.

Dedysh, S. N., Pankratov, T.A., Belova, S. E., Kulichevskaya, I.S., and Liesack, W. (2006). Phylogenetic analysis and in situ identification of bacteria community composition in an acidic *Sphagnum* peat bog. *Appl. Environ. Microbiol.* 72, 2110–2117.

Dedysh, S.N. (2009). Exploring methanotrophs diversity in acidic northern wetlands: molecular and cultivation-based studies. *Microbiology* 78, 655–669.

Dedysh, S.N. (2011). Cultivating uncultured bacteria from northern wetlands: knowledge gained and remaining gaps. Front. Microbiol. 2:184. http://dx.doi.org/10.3389/fmicb.2011.00184.

Deng, S., Popova, I. (2011). Carbohydrate hydrolases. In: Dick R.P. (ed.) Methods of soil enzymology. Soil Science Society of America, Inc., Madison, pp 185–209

Denison, R. F., & Kiers, E. T. (2011). Life histories of symbiotic rhizobia and mycorrhizal fungi. Curr Biol 21, R775–R785. Elsevier Ltd.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., and Andersen, G. L. (2006). Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. Appl Environ Microbiol 72:5069-72.

Dhal, P.K., Islam, E., Kazy, S.K., Sar, P. (2011). Culture-independent molecular analysis of bacterial diversity in uranium-ore/-mine waste-contaminated and non-contaminated sites from uranium mines. 3 Biotech. 1 (4), 261e272.

Dion, P., Nautiyal, C.S. (2008). Extreme views on prokaryote evolution. Microbiol. Extreme Soils, 45–70.

Drake, H.L., Horn, M.A., Wust, P.K. (2009). Intermediary ecosystem metabolism as a main driver of methanogenesis in acidic wetland soil. Environ. Microbiol. Rep. 1:307–318. http://dx.doi.org/10.1111/j.1758-2229.2009.00050.x.

Dray, S., and Dufour, A.B. (2007). The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software. 22(4): 1-20.

Dunfield, P.F., Yuryev, A., Senin, P., Smirnova, A.V., Stott, M.B., Hou, S., Saw, J.H., Zhou, Z., Ren, Y., Wang, J., Mountain, B.W., Crowe, M.A., Weatherby, T.M., Bodelier, P.L.E., Liesack, W., Feng, L., Wang, L., Alam, M. (2007). Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. Nature 450:879–882

Dunn, C., Jones, T., Girard, A., and Freeman, C. (2014). "Methodologies for Extracellular Enzyme Assays from Wetland Soils." *Wetlands* 34, no. 1: 9–17. doi:10.1007/s13157-013-0475-0.

Fan, M., Lin, Y., Huo, H., Liu, Y., Zhao, L., Wang, E., Chen, W., and Wei, G. (2016). "Microbial Communities in Riparian Soils of a Settling Pond for Mine Drainage Treatment." *Water Research* 96: 198–207. doi:10.1016/j.watres.2016.03.061.

Feris, K., Ramsey, P., Frazar, C., Moore, J.N., Gannon, J.E., Holben, W.E. (2003). Differences in hyporheic-zone microbial community structure along a heavy metal contamination gradient. Appl. Environ. Microbiol. 69 (9), 5563e5573.

Fierer, N., and Jackson, R. B. (2006). "The Diversity and Biogeography of Soil Bacterial Communities." *Proceedings of the National Academy of Sciences* 103, no. 3: 626–31. doi:10.1073/pnas.0507535103.

Freedman, B., and Hutchinson, T.C. (1980a). Pollutant inputs from the atmosphere and accumulations in soils and vegetation near a nickel-copper smelter at Sudbury, Ontario, Canada. *Can. J. Bot*. 58. Pg. 108-132.

Freedman, B., and Hutchinson, T.C. (1980b). Long-term effects of smelter pollution at Sudbury, Ontario, on forest community composition. *Can. J. Bot.* 58: 19. Pg. 2123-2140.

Freeman C., Liska G., Ostle N.J., Lock M.A., Hughes S. Reynolds B. and Hudson J. (1997). Enzymes and biogechemical cycling in wetlands during simulated drought. *Biogeochemistry.* 39: 177-187.

Freeman, C., Evans, C.D., Monteith, D.T., Reynolds, B., Fenner, N. (2001). Export of organic carbon from peat soils. Nature 412, 785-787.

Freeman, C., Fenner, N., Ostle, N.J., Kang, H., Dowrick, D.J., Reynolds, B., Lock, M.A., Sleep, D., Hughes, S., Hudson, J. (2004). Export of Dissolved Organic Carbon from peatlands under elevated CO2 levels. Nature 430, 195e198.

Garcia, J. L., Patel, B.K.C., and Ollivier, B. (2000). Taxonomic, phylogenetic, and ecological diversity of methanogenic Archaea. Anaerobe. 6(4): 205–226.

Gauci, V., Matthews, E., Dise, N., Walter, B., Koch, D., Granberg, G., and Vile, M. (2004). Sulfur pollution suppression of the wetland methane source in the 20th and 21st centuries. Proc. Natl. Acad. Sci. 101(34): 12 583–12 587.

Gignac, L.D., Beckett, P.J. (1986). The effect of smelting operations on peatlands near Sudbury, 61 Ontario, Canada. Can. J. Bot. 64, 1138–1147

Gignac, L.D., Vitt, D.H., and Bayley, S. (1991). Bryophyte Response Along Ecological and Climatic Gradients. *Vegetation.* 93. Pg. 29-45.

Gorham, E., Bayley, S. E., and Schindler, D.W. (1984). Ecological effects of acid deposition upon peatlands: a neglected field in" acid-rain" research. *Can. J. Fish. Aquat. Sci*. 41: 8. Pg. 1256-1268.

Gorham, E. (1991). Northern peatlands: role in the carbon cycle and probable responses to climatic warming. Ecological Applications, 1, 182–195.

Gunn, J.M. (1995). Restoration and recovery of an industrial region. New York: Springer- Verlag.

Gunn, J., Keller, W., Negusanti, J., Potvin, R., Beckett, P., and Winterhalder, K. (1995). Ecosystem recovery after emission reductions: Sudbury, Canada. *Water, Air and Soil Pollution.* 85. Pg. 1783-1788.

Gupta, V., Smemo, K. A., Yavitt, J. B., & Basiliko, N. (2012). Active methanotrophs in two contrasting North American peatland ecosystems revealed using DNA-SIP. *Microbial ecology*, *63*(2), 438-445.

Hazlett, P.W., Rutherford, G.K., VanLoon, G.W. (1984). Characteristics of soil profiles affected by smelting of nickel and copper at Coniston, Ontario, Canada. Geoderma 32, 273–285.

Hanson, R.S., Hanson, T.E. (1996). Methanotrophic bacteria. Microbiol Rev 60:439–471

Haouari, O., Fardeau, M.-L., Cayol, J.-L., Fauque, G., Casiot, C., Elbaz-Poulichet, F., Hamdi, M., and Ollivier, B. (2008). Thermodesulfovibriohydrogeniphilus sp. nov., a new thermophilic sulphate-reducing bacterium isolated from a Tunisian hot spring. Syst. Appl. Microbiol. 31, 38–42.

Hein, R., Crutzen, P.J., and Heinmann, M. (1997). An inverse modeling approach to investigate the global atmospheric methane cycle. *Global Biogeochem. Cycles* 11, 43–76.

Heising, S., & Schink, B. (1998). Phototrophic oxidation of ferrous iron by a Rhodomicrobium vannielii strain. Microbiology 144: 2263–2269.

Hervé, M. (2015). RVAideMemoire: Diverse Basic Statistical and Graphical Functions. R package version 0.9-45-2. http://CRAN.R-project.org/package=RVAideMemoire

Holden, J., Burt, T.P. (2003). Hydrological studies on blanket peat: the significance of the acrotelm-catotelm model. Journal of Ecology 91, 86–102.

Hutchinson, T. C., and Whitby, L. M. (1974). Heavy metal pollution in the Sudbury mining and smelting region of Canada, I. Soil and vegetation contamination by nickel, copper and other metals. Environ. Conserv. 1: 2. Pg. 123.

Hutchinson, T.C., Whitby, L.M. (1977). The effects of acid rainfall and heavy metal particulates on a boreal forest ecosystem near the Sudbury smelting regions of Canada. Water Air Soil Pollut. 7, 421–438.

IBM Corp. (Released 2012). IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

Ingram, H.A.P. (1978). Soil layers in mires: function and terminology. Journal of Soil Science, 29, 224–227.

Ingram, H.A.P. (1983). Hydrology. In: Gore, A.J.P. (Ed.), Ecosystems of the World 4A, Mires: swamp, bog, fen and moor. Elsevier, Oxford, pp. 67–158.

Joosten, H., Clarke, D. (2002). Wise use of Mires and Peatlands, a Framework for Decision Making. International Mire Conservation Group & International Peat Society.

Kamal, S., and Varma, A. (2008). Peatland microbiology. In Dion, P. and Nautiyal, C. S. (eds.) Microbiology of Extreme Soils. Soil Biology 13. Springer-Verlag, Berlin Heidelberg. pp. 177–203.

Kang, H.J., Freeman, C., Park, S.S., Chun, J. (2005). N-Acetylglucosaminidase activities in wetlands: a global survey. Hydrobiologia 532

Kang, S., Van Nostrand, J.D., Gough, H.L., He, Z.L., Hazen, T.C., Stahl, D.A., Zhou, J.Z. (2013). Functional gene arrayebased analysis of microbial communities in heavy metals-contaminated lake sediments. FEMS Microbiol. Ecol. 86 (2), 200e214.

Keller, W., Yan, N.D., Gunn, J.M., Heneberry, J. (2007). Recovery of acidified lakes: lessons from Sudbury, Ontario, Canada. Water Air Soil Pollut. Focus 7, 317-322.

Kersters, K., De Vos, P., Gillis, M., Swings, J., Vandamme, P., & Stackebrandt, E. (2006). Introduction to the Proteobacteria. The Prokaryotes, Vol. 5 (Dwarkin M, Falkow S, Rosenberg E, Schleifer KH & Stackebrandt E, eds), pp. 3–37. Springer, New York, NY.

Klose, S., Bilen, S., Tabatabai, M.A., Dick, W.A. (2011). Sulfur cycle enzymes. In: Dick RP (ed) Methods of soil enzymology. Soil Science Society of America, Wisconsin

Konhauser, K.O. (2009). *Introduction to geomicrobiology*. John Wiley & Sons.

Kumar, S., Stecher, G., and Tamura, K. (2015). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution (submitted).

Lai, D.F.Y. (2009). Methane dynamics in northern peatlands: a review. Pedosphere 19:409–421

Limpens, J., Berendse, F., Blodau, C., Canadell, J.G., Freeman, C., Holden, J., Roulet, N., Rydin, H., Schaepman-Strub, G. (2008). Peatlands and the carbon cycle: from local processes to global implications—a synthesis. Biogeosciences 5:1475–1491. http://dx.doi.org/10.5194/bg-5-1475-2008.

Lin, X., Tfaily, M. M., Steinweg, J. M., Chanton, P., Esson, K., Yang, Z. K., Chanton, J. P., Cooper, W., Schadt, C. W., and Kostka, J. E. (2014a). "Microbial Community Stratification Linked to Utilization of Carbohydrates and Phosphorus Limitation in a Boreal Peatland at Marcell Experimental Forest, Minnesota, USA." *Applied and Environmental Microbiology* 80, no. 11: 3518–30. doi:10.1128/AEM.00205-14.

Lin, X., Tfaily, M. M., Green, S. J., Steinweg, J. M., Chanton, P., Imvittaya, A., Chanton, J. P., Cooper, W., Schadt, C., and Kostka, J. E. (2014b). "Microbial Metabolic Potential for Carbon Degradation and Nutrient (Nitrogen and Phosphorus) Acquisition in an Ombrotrophic Peatland." *Applied and Environmental Microbiology* 80, no. 11: 3531–40. doi:10.1128/AEM.00206-14.

Luke, S., Preston, M. D., Basiliko, N., & Watmough, S. A. (2015). Microbial Communities, Biomass, and Carbon Mineralization in Acidic, Nutrient-Poor Peatlands Impacted by Metal and Acid Deposition. *Water, Air, & Soil Pollution*, *226*(2), 1-13.

Martínez-Cortizas, A., Pontevedra-Pombal, X., Garcia-Rodeja, E., Novoa-Munoz, J.C., Shotyk, W. (1999). Mercury in a Spanish peat bog: Archive of climate change and atmospheric metal deposition. Science 284, 939-942.

Marx, M.C., Wood, M., Jarvis, S.C. (2001). Amicroplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol Biochem 33:1633–1640

Masella, A.P., Bartram, A.K., Truszkowski, J.M., Brown, D.G., & Neufeld, J.D. (2012). *PANDAseq: paired-end assembler for illumina sequences*, BioMed Central: The Open Acces Publisher. Retrieved at October 10, 2012, from the website temoa: Open Educational Resources (OER) Portal at http://www.temoa.info/node/440111

Matthews, E., Fung, I. (1987). Methane emissions from natural wetlands: global distribution, area, and environmental characteristics of sources. Global Biogeochem Cycles 1: 61–86.

McCaig, A.E., Glover, L.A., Prosser, J.I. (1999). Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl. Environ. Microbiol. 65, 1721–1730.

McMurdie and Holmes. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE. 8(4):e61217

Meadows, M., Watmough, S.A. (2012). An assessment of long-term risks of metal in Sudbury: A critical loads approach. Water Air Soil Pollut. 223, 4343-4354.

Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol. J. Environ. Stud.* **15**: 523-530.

Mitsch, W., and Gosselink, J.G. (2000). Wetlands (3erd ed.). New York: John Wiley & Sons

Monet, S. (2013). Greater Sudbury Natural Heritage Background Report. City of Greater Sudbury, Ontario, Canada.

Moore, P.D. and Bellamy, D.J. (1974). Peatlands. London: Elek science.

Morales, S.E., Mouser, P.J., Ward, N., Hudman, S.P., Gotelli, N.J., Ross, D.S., and Lewis, T.A. (2006). Comparison of bacterial communities in New England *Sphagnum* bogs using terminal restriction fragment length polymorphism. *Microb. Ecol.* 52, 34–44.

Murrell, J. C., McDonald, I. R., & Gilbert, B. (2000). Regulation of expression of methane monooxygenases by copper ions. *Trends in microbiology*, *8*(5), 221-225.

Myers, B., Webster, K. L., McLaughlin, J.W., and Basiliko, N. (2012). Microbial activity across a boreal peatland nutrient gradient: the role of fungi and bacteria. *Wetlands Ecol. Man.* 20, 77–88. doi:10.1007/s11273-011-9242-2

Nriagu, J.O., Wong, H.K.T., Lawson, G., Daniel, P. (1998). Saturation of ecosystems with toxic metals in Sudbury basin, Ontario, Canada. Sci. Total Environ. 223, 99-117.

Oksanen J., Guillaume B.F., Kindt R., Legendre P., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P., Henry M., Stevens H., and Wagner H. (2015). vegan: Community Ecology Package. R package version 2.2-1. http://CRAN.R-project.org/package=vegan

Olid, C., Diego, D., Garcia-Orellana, J., Cortizas, A. M., Klaminder, J. (2016). Modeling the downward transport of 210Pb in Peatlands: Initial penetration-constant rate of supply (IP-CRS) model. Science of the Total Environment 541, 1222-1231.

Op den Camp, H.J.M., Islam, T., Stott, M.B., Harnangi, H.R., Hynes, A., Schouten, S., Jetten, M.S.M., Brikenland, N.-K., Pol, A., Dunfield, P.F. (2009). Environmental, genomic and taxonomic perspectives on methanotrophic Verrucomicrobia. Environmental Microbiology Reports 1, 293e306.

Oshrain, R.L., Wiebe, W.J. (1979). Arylsulfatase activity in salt marsh soils. Appl Environ Microbiol 38:337–340

Pamer, J. L. (2008). Characterization of Anthropogenic Pollution in Sphagnum Peat from the Sudbury Region: Sudbury Region Peatlands as Archives of Atmospheric Base Metal Deposition (unpublished MSc. Thesis)

Panikov, N.S. (1999). Fluxes of CO2 and CH4 in high latitude wetlands: measuring, modeling and predicting response to climate change. Polar Res 18:237–244.

Pankratov, T.A., Belova, S.E., Dedysh, S.N. (2006). Evaluation of the phylogenetic diversity of prokaryotic microorganisms in Sphagnum peat bogs by means of fluorescence in situ hybridization (FISH). Microbiology 74, 722e728.

Pelletier, L., Moore, T. R., Roulet, N. T., Garneau, M., and Beaulieu-Audy, V. (2007). Methane fluxes from three peatlands in the La Grande Rivi`ere watershed, James Bay lowland, Canada. J. Geophys. Res. 112: G01018, doi:10.1029/2006JG000216.

Ploner, A., (2014). Heatplus: Heatmaps with row and/or column covariates and colored clusters. R package version 2.12.0.

Post, W.M., Emanuel, W.R., Zinke, P.L. & Stangenberger, A.G. (1982). Soil carbon pools and world life zones. Nature, 298, 156–159.

Poulsen, M., Schwab, C., Jensen, B.B., Engberg, R.M., Spang, A., Canibe, N., Hojberg, O., Milinovich, G., Fragner, L., Schleper, C., Weckwerth, W., Lund, P., Schramm, A., Urich, T. (2013). Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen. Nature Communications 4.

Press, M.C., Henderson, J., Lee, J.A. (1985). Arylsulfatase activity in peat in relation to acidic deposition. Soil Biol Biochem 17:99–103

Puglisi, E., Zaccone, C., Cappa, F., Cocconcelli, P.S., Shotyk, W., Trevisan, M., and Miano, T.M. (2014). “Changes in Bacterial and Archaeal Community Assemblages along an Ombrotrophic Peat Bog Profile.” *Biology and Fertility of Soils* 50, no. 5 815–26. doi:10.1007/s00374-014-0902-2.

R Core Team. (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rastogi, G., Osman, S., Vaishampayan, P.A. (2010). Microbial diversity in uranium mining impacted soils as revealed by high-density 16S microarray and clone library. Microbiol Ecol; 59:94–108.

Reddy, K.R., DeLaune, R.D. (2008). Biogeochemistry of Wetlands. CRC press, New York, NY.

Reed, S.C., Cleveland, C.C., Townsend, A.R. (2013). Relationships among phosphorus, molybdenum and free-living nitrogen fixation in tropical rain forests: results from observational and experimental analyses. Biogeochemistry114:1–13. http://dx.doi.org/10.1007/s10533-013-9835-3.

Roberts, D.W. (2015). labdsv: Ordination and Multivariate Analysis for Ecology. R package version 1.7-0. http://CRAN.R-project.org/package=labdsv

Romanowicz, K.J., Kane, E.S., Potvin, L.R., Daniels, A.L., Kolka, R.K., and Lilleskov, E.A. (2015). "Understanding Drivers of Peatland Extracellular Enzyme Activity in the PEATcosm Experiment: Mixed Evidence for Enzymic Latch Hypothesis." *Plant and Soil* 397, no. 1–2: 371–86. doi:10.1007/s11104-015-2746-4.

Rydin, H., Jeglum, J. (2006). The Biology of Peatlands. Oxford University Press, New York

SARA Group. (2001). Sudbury Area Risk Assessment. Chapter 7.0: The 2001 Soil Survey. Accessed on line. October 2016. http://www.sudburysoilsstudy.com/EN/media/Volume_I/SSS_Vol_I_Chapter_7_The2001SoilSurvey_FINAL_Jan2008.pdf

Schiff, S., Mewhinney, E., Elgood, R., Warner, B., Dillon, P. and Trumbore, S. (1998). Precambrian Shield Wetlands: Hydrologic control of the sources and export of dissolved organic matter. *Climatic Change*. 40:2. Pg. 167-188.

Semkin, R.G. and Kramer, J.R. (1976). *Can. Mineral*. 14. Pg. 73-90.

Shotyk, W. (1996). Peat bog archives of atmospheric metal deposition: geochemical evaluation of peat profiles, natural variations in metal concentrations, and metal enrichment factors. Environmental Reviews, 4(2),149-183.

Shotyk, W., Weiss, D., Appleby, P.G., Cheburkin, A.K., Frei, R., Gllor, M., Kramers, J.D., Reese, S., Van Der Knaap, W.O. (1998). History of atmospheric lead deposition since 12,370 14C yr BP from a peat bog, Jura Mountains, Switzerland. Science 281:1635–1640

Sinsabaugh, B. (2009a). http://enzymes.nrel.colostate.edu/enzymes/protocol/HydrolaseEnzymeAssaysProtocol11.14.2000.pdf

Sinsabaugh, R.L., Hill, B.H., Shah, J.J.F. (2009b). Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795–798

Slack, N. G., Vitt, D. H., and Horton, D. G. (1980). Vegetation gradients of minerotrophically rich fens in western Alberta. *Canadian Journal of Botany*. 58. Pg. 330–350.

Smith, L.C., MacDonald, G.M., Velichko, A.A., Beilman, D.W., Borisova, O.K. (2004). Siberian peatlands a net carbon sink and global methane source since the early Holocene. Science 303: 353–356.

Spitzer, K., Danks, H.V. (2006). Insect biodiversity of boreal peat bogs. Annu Rev Entomol 51:137–161

Souter, L., and Watmough, S.A. (2016). "The Impact of Drought and Air Pollution on Metal Profiles in Peat Cores." *Science of The Total Environment* 541: 1031–40. doi:10.1016/j.scitotenv.2015.09.137.

Suyama, Y., Gunnarsson, U., Parducci, L. (2008). Analysis of short DNA fragments from Holocene peatmoss samples. The Holocene 18: 1003–1006

Szkokan-Emilson, E.J., Wesolek, B.E., and Gunn, J. M. (2011). Terrestrial organic matter as subsidies that aid in the recovery of macroinvertebrates in industrially damaged lakes. *Ecological Applications*. 21: 6. Pg. 2082-2093.

Szkokan-Emilson, E.J., Kielstra, B., Watmough, S.A., and Gunn, J.M. (2013). Drought-induced release of metals from peatlands in watersheds recovering from historical metal and sulphur deposition. *Biogeochemistry*. 1-15. Published online, 23 October 2013.

Szkokan-Emilson, E.J., Watmough, S.A., Gunn, J.M. (2014). Wetlands as long-term sources of metals to receiving waters in mining-impacted landscapes. Environ. Pollut. 192, 91–103.

Szumigalski, A.R., and Bayley, S.E. (1996). Decomposition along a bog to rich fen gradient in central Alberta, Canada. *Can. J. Bot*. 74. Pg. 573-581.

Tan, G.L., Shu, W.S., Zhou, W.H., Li, X.L., Lan, C.Y., Huang, L.A. (2009). Seasonal and spatial variations in microbial community structure and diversity in the acid stream draining across an ongoing surface mining site. FEMS Microbiol. Ecol. 70(2), 277e285.

Taylor, G.J., Crowder, A.A. (1983). Accumulation of atmospherically deposited metals in wetland soils of Sudbury, Ontario. Water Air Soil Pollut. 19, 29-42.

Turner, D.S., Amon, J.P., Schnuble, R.M., Friese, C.F. (2000). Mycorrhizal fungi associated with plants in ground-water fed wetlands. Wetlands 20:200–204

Turner, B.L., Baxter, R., Whitton, B.A. (2002). Seasonal phosphatase activity in three characteristic soils of the English uplands polluted by longterm atmospheric nitrogen deposition. Environ Pollut 120

Tyler, G., (1990). Bryophytes and heavy metals: a literature review. Bot. J. Linn. Soc. 104, 231- 253.

Urbanová, Z., and Bárta J. (2016). "Effects of Long-Term Drainage on Microbial Community Composition Vary between Peatland Types." *Soil Biology and Biochemistry* 92: 16–26. doi:10.1016/j.soilbio.2015.09.017.

Van Alphen, M. (1999). Atmospheric heavy metal deposition plumes adjacent to a primary lead- zinc smelter. Sci. Total Environ. 236, 119-34.

Van Breemen, N. (1995). How Sphagnum bogs down other plants. Trends in Ecology & Evolution, 10, 270–275.

Vecherskaya, M., Dijkema, C., Saad, H.R., Stams, A.J.M. (2009). Microaerobic and anaerobic metabolism of a Methylocystis parvus strain isolated from a denitrifying bioreactor. Environ Microbiol Rep 1: 442–449.

Vitt, D.H., Halsey, L.A., Bauer, I.E., & Campbell, C. (2000). Spatial and temporal trends in carbon storage of peatlands of continental western Canada through the Holocene. Canadian Journal of Earth Sciences/Revue Canadienne des Sciences de la Terre, 37, 683–693.

Vitousek, P.M., Hobbie, S. (2000). Heterotrophic nitrogen fixation in decomposing litter: patterns and regulation. Ecology 81:2366–2376. http://dx.doi.org/10.1890/00129658(2000)081[2366:HNFIDL]2.0.CO;2.

Vitousek, P.M., Menge, D.N., Reed, S.C., Cleveland, C.C. (2013). Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368:20130119. http://dx.doi.org/10.1098/rstb.2013.0119.

Von Post, L. (1922). Sveriges geologiska undersöknings torvinvenstering och några av dess hittills vaanna resultant. Svenska Mosskulturfören. Tidskr. 36:1–27.

Wahlen, M. (1993). The global methane cycle. Ann Rev Earth Planet Sci 21:407–426.

Waksman, S.A., Purvis, E.R. (1932). The microbiological population of peat. Soil Science 34, 95e109.

Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007). Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Appl Environ Microbiol. 73(16):5261-7.

Warnes, G. R., Bolker, B., Bonebakker, L., Gentleman, R., Liaw, W. H. A., Lumley, T., Maechler, M., Magnusson, A., Moeller, S., Schwartz, M., and Venables, B. (2015). gplots: Various R Programming Tools for Plotting Data. R package version 2.17.0. http://CRAN.R-project.org/package=gplots

Wells, E. D., and Zoltai, S. (1985). The Canadian System of Wetland Classification and its Application to Circum Boreal Wetlands. *Aquilo Ser. Bot*. 21. Pg. 45-52.

Wells, J.V., Roberts, D., Lee, P., Cheng, R., Darveau, M. (2011). A forest of blue: Canada's Boreal Forest, the world's waterkeeper. Pew Environmental Group, Washington, USA

Whalen, S. C. (2005). Biogeochemistry of methane exchange between natural wetlands and the atmosphere. Environ. Eng. Sci. 22(1): 73–94.

Wickham, H. (2009). ggplot2: elegant graphics for data analysis. Springer New York.

Williams, R.T., Crawford, R.L. (1983). Microbial diversity in Minnesota peatlands. Microbial Ecology 9, 201e214

Winsborough, C. L., and Basiliko, N. (2010). Fungal and bacterial activity in northern peatlands. *Geomicrobiol.J.* 27, 315–320.doi: 10.1080/01490450903424432

Winterhalder, K. (1996). Environmental degradation and rehabilitation of the landscape around Sudbury, a major mining and smelting area. *Environ. Rev.* **4**: 185-224.

Yavitt, J.B., Williams, C.J. & Wieder, R.K. (1997). Production of methane and carbon dioxide in peatland ecosystems across North America: Effects of temperature, aeration, and organic chemistry of peat. Geomicrobiol. J. 14: 299–316

Yrjala, K.I.M., Tuomivirta, T., Juottonen, H., Putkinen, A., Lappi, K., Tuittila, E.-S., Penttil€A, T., Minkkinen, K., Laine, J., Peltoniemi, K., Fritze, H. (2011). CH4 production and oxidation processes in a boreal fen ecosystem after long-term water table drawdown. Global Change Biology 17, 1311e1320.

Zoltai, S. C., and Vitt, D.H. (1995). Canadian Wetlands: Environmental gradients and classification. *Vegetatio*. 118. Pg. 131-137.

# 7 Appendix

## 7.1 von Post classification of peat

Von Post was evaluated in the field by squeezing the peat samples and observing the color of the solution, and the proportion of the original sample that remained after squeezing, along with the nature of the fibers.

**Table 7.16-** Von Post Scale of Humification (source Jamie Lamit, 2014)

| THE VON POST SCALE | DESCRIPTION |
|---|---|
| H1 | Completely un-decomposed peat which, when squeezed, releases almost clear water. Plant remains easily identifiable. No amorphous material present. |
| H2 | Almost entirely un-decomposed peat which, when squeezed, releases clear or yellowish water. Plant remains still easily identifiable. No amorphous material present. |
| H3 | Very slightly decomposed peat which, when squeezed, releases muddy brown water, but from which no peat passes between the fingers. Plant remains still identifiable, and no amorphous material present. |
| H4 | Slightly decomposed peat which, when squeezed, releases very muddy dark water. No peat is passed between the fingers but the plant remains are slightly pasty and have lost some of their identifiable features. |
| H5 | Moderately decomposed peat, which when squeezed, releases very "muddy" water with a very small amount of amorphous granular peat escaping between the fingers. The structure of the plant remains is quite indistinct although it is still possible to recognize certain features. The residue is very pasty. |
| H6 | Moderately highly decomposed peat with a very indistinct plant structure. When squeezed, about one-third of the peat escapes between the fingers. The residue is very pasty but shows the plant structure more distinctly than before squeezing. |
| H7 | Highly decomposed peat. Contains a lot of amorphous material with very faintly recognizable plant structure. When squeezed, about one-half of the peat escapes between the fingers. The water, if any is released, is very dark and almost pasty. |
| H8 | Very highly decomposed peat with a large quantity of amorphous material and very indistinct plant structure. When squeezed, about two-thirds of the peat escapes between the fingers. A small quantity of pasty water may be released. The plant material remaining in the hand consists of residues such as roots and fibers that resist decomposition. |
| H9 | Practically fully decomposed peat in which there is hardly any recognizable plant structure. When squeezed it is a fairly uniform paste. |
| H10 | Completely decomposed peat with no discernible plant structure. When squeezed, all the wet peat escapes between the fingers. |

## 7.2 Moisture classification of peat

Moisture was evaluated in the field by squeezing the peat samples and observing the amount of liquid produced.

**Table 7.17-** Moisture Scale (source Jamie Lamit, 2014)

| PEAT MOISTURE SCALE | DESCRIPTION |
|---|---|
| B1 | Dry peat |
| B2 | Low moisture content |
| B3 | Moderate moisture content |
| B4 | High moisture content |
| B5 | Very high moisture content |

## 7.3 Site Characteristics

**Table 7.18**– Description of study site locations and summary characteristics including microtopography and moss richness from June-August 2014.

| Peatland | Latitude | Longitude | Elevation (m) | Distance from Copper Cliff (km) | Richness of Sphagnum moss | Richness of other mosses | Micro-topography |
|---|---|---|---|---|---|---|---|
| Cartier1 (Cart1) | 46.3976 | 81.3123 | 423.6720 +/- 3.0480 | 55.1 | 3 | 0 | Hummocks and hollow |
| Cartier2 (Cart2) | 46.3777 | 81.3120 | 423.6720 +/- 3.0480 | 55.0 | 3 | 0 | Lawn/mid-lawn |
| Ashigami (Ashi) | 46.6458 | 80.6144 | 307.2384 +/-3.6576 | 39.15 | 2 | 1 | Floating mats/lawn |
| Rockcut (Rock) | 46.43644 | 80.55598 | 326.4408 +/- 3.0480 | 29.86 | 1 | 0 | Hummocks and hollows |
| Broder (Brod) | 46.2373 | 80.5807 | 267.0050 +/- 3.0480 | 11.46 | 1 | 1 | Hummocks and hollows |
| Crowley (Craw) | 46.3885 | 80.9719 | 277.6730 +/- 0.9144 | 12.45 | 0 | 1 | Hummocks and hollows |
| Daisy-I (Dais) | 46.4549 | 80.8825 | 249.3260 +/- 2.7432 | 13.93 | 1 | 1 | Lawn with patchy moss hummocks |
| Long Lake (Long) | 46.3704 | 81.066 | 286.5120 +/- 0.9144 | 11.78 | 1 | 0 | Lawn |
| Clearwater (Crea) | 46.2216 | 81.0371 | 334.6704 +/- 3.0480 | 12.13 | 0 | 1 | Shallow hummocks |
| Laurentian (Laur) | 46.4553 | 80.9675 | 294.7420 +/- 3.3528 | 7.53 | 0 | 1 | Shallow hummocks and hollows |
| Whitson (Whit) | 46.35401 | 80.59496 | 299.0090 +/- 3.3528 | 12.18 | 1 | 0 | Lawn with patchy sphagnum hummocks |

**Table 7.19-** Peatland characteristics by plots showing site direction from Copper Cliff, peatland type, plant characcteristics and peat chemistry

| Sites | Plots | Direction | Peatland type | Microtopography | Maximum Height Vegetation (m) | Canopy cover | Pore water pH | Pore water Conductivity +/- 4 | Watertable level (cm) |
|---|---|---|---|---|---|---|---|---|---|
| Cartier 1 | 1 | NNW | fen (treed) | sphagnum hummock/hollow | 0.7-0.9 | 5 | 3.69 | 55 | 25 |
| | 2 | | | | | <5 | 3.70 | 34 | 25 |
| | 3 | | | | | 10 | 3.63 | 37 | 18 |
| Cartier 2 | 1 | NNW | fen (lawn) | sphagnum and sedges | 0.3-0.5 | 0 | 3.67 | 85 | 5 |
| | 2 | | | | | 0 | 3.63 | 44 | 5 |
| | 3 | | | | | 0 | 3.76 | 37 | 5 |
| Ashigami | 1 | NE | poor fen, floating | floating mats/lawn | 1.0-1.5 | 0 | 5.42 | 32 | 5 |
| | 2 | | | | | 0 | 5.11 | 21 | 13 |
| | 3 | | | | | 0 | 5.12 | 17 | 8 |
| Rockcut | 1 | NNE | poor fen | hummocks and hollows | 0.5-1.0 | 0 | 4.02 | 19 | 12 |
| | 2 | | | | | 0 | 4.00 | 25 | 20 |
| | 3 | | | | | 0 | 4.30 | 26 | 20 |
| Broder | 1 | SSE | intermediate fen | hummock and hollow | 0.5-0.75 | 0 | 4.73 | 23 | 0 |
| | 2 | | | | | 0 | 4.45 | 13 | 0 |
| | 3 | | | | | 0 | 4.49 | 14 | 0 |
| Long Lake | 1 | S | poor fen | lawn | 0.9-1.75 | 0 | 4.88 | 30 | 4 |
| | 2 | | | | | 25 | 5.24 | 53 | 3 |
| | 3 | | | | | 0 | 4.68 | 30 | 0 |
| Crowley | 1 | SSE | poor fen | edge of hummock and hollow | 0.5-1.0 | 0 | 4.26 | 36 | 16 |
| | 2 | | | | | 0 | 4.30 | 30 | 9 |
| | 3 | | | | | 0 | 4.12 | 21 | 5 |
| Daisy I | 1 | SEE | poor fen | lawn with patchy moss hummocks | 0.68-0.81 | 0 | 4.37 | 45 | 8 |
| | 2 | | | | | 0 | 4.40 | 39 | 7 |
| | 3 | | | | | 0 | 4.72 | 30 | 15 |
| Lake Laurentian | 1 | SE | poor fen | shallow humocks and hollow | 0.6-0.7 | 0 | 4.07 | 49 | 15 |
| | 2 | | | | | 0 | 4.28 | 44 | 12 |
| | 3 | | | | | 0 | 4.47 | 43 | 12 |
| Clearwater | 1 | S | poor fen | shallow hummocks | 0.5-0.67 | 0 | 3.77 | 48 | 10 |
| | 2 | | | | | 0 | 4.16 | 75 | 7 |
| | 3 | | | | | 0 | 3.70 | 67 | 7 |
| Whitson | 1 | NNE | poor to intermediate fen | lawn with patchy sphagnum hummocks | 0.5-0.8 | 0 | 5.54 | 41 | 22 |
| | 2 | | | | | 0 | 5.10 | 37 | 19 |
| | 3 | | | | | 0 | 5.08 | 40 | 14 |

**Table 7.20**- Peatlands chemistry, including temperature, von Post, moisture and pH across sites and depths.

| Sites | Depth | Core Temp °C | Von Post score | Moisture score | Peat pH |
|---|---|---|---|---|---|
| Ashigami | 0 | 15.9 | 2 | 4 | 5.13 |
| | 0 | 17.9 | 4 | 4 | 7.28 |
| | 0 | 15.1 | 3 | 4 | 5.24 |
| | 10 | 15.6 | 3 | 5 | 4.89 |
| | 10 | 15 | 4 | 5 | 6.03 |
| | 10 | 14.5 | 4 | 5 | 4.77 |
| | 30 | 15.7 | 7 | 5 | 4.81 |
| | 30 | 14.7 | 7 | 5 | 4.65 |
| | 30 | 16.4 | 7 | 5 | 4.72 |
| | 60 | 14.7 | 8 | 5 | 4.8 |
| | 60 | 14 | 7 | 5 | 4.81 |
| | 60 | 15.7 | 7 | 5 | 4.93 |
| Broder | 0 | 17.6 | 3 | 5 | 4.77 |
| | 0 | 17.3 | 3 | 5 | 5.87 |
| | 0 | 16.4 | 4 | 5 | 5.78 |
| | 10 | 17.2 | 4 | 5 | 4.87 |
| | 10 | 18 | 4 | 5 | 4.62 |
| | 10 | 17.6 | 5 | 5 | 4.69 |
| | 30 | 16.9 | 7 | 5 | 5.34 |
| | 30 | 16.3 | 7 | 5 | 5.15 |
| | 30 | 16.5 | 7 | 5 | 5.18 |
| | 60 | 15 | 9 | 5 | 5.47 |
| | 60 | 10.5 | 8 | 5 | 5.56 |
| | 60 | 15.5 | 8 | 5 | 5.6 |
| Cartier 1 | 0 | 12.1 | 3 | 3 | 3.57 |
| | 0 | 10.1 | 3 | 3 | 3.54 |
| | 0 | 12.3 | 3 | 4 | 3.49 |
| | 10 | 12.1 | 4 | 4 | 3.53 |
| | 10 | 10.6 | 4 | 4 | 3.52 |
| | 10 | 11.3 | 4 | 4 | 3.4 |
| | 30 | 11.6 | 5 | 5 | 3.55 |
| | 30 | 10.2 | 5 | 5 | 3.55 |
| | 30 | 9.6 | 7 | 5 | 3.48 |
| | 60 | 9.6 | 8 | 5 | 3.51 |
| | 60 | 7.8 | 8 | 5 | 3.51 |
| | 60 | 7.7 | 7 | 5 | 3.45 |
| Cartier 2 | 0 | 15.4 | 2 | 5 | 3.73 |
| | 0 | 15.5 | 3 | 5 | 3.56 |
| | 0 | 15.8 | 3 | 5 | 3.55 |
| | 10 | 16.2 | 3 | 5 | 3.61 |
| | 10 | 16.8 | 4 | 5 | 3.46 |
| | 10 | 17.1 | 4 | 5 | 3.55 |
| | 30 | 14.1 | 5 | 5 | 3.53 |
| | 30 | 15.1 | 5 | 5 | 3.5 |
| | 30 | 15.2 | 5 | 5 | 3.5 |
| | 60 | 12.7 | 5 | 5 | 3.56 |
| | 60 | 13.5 | 5 | 5 | 3.38 |
| | 60 | 13.2 | 5 | 5 | 3.44 |
| Long Lake | 0 | 17.7 | 4 | 5 | 6.6 |
| | 0 | 16.4 | 4 | 5 | 6.52 |
| | 0 | 18.1 | 4 | 5 | 5.62 |
| | 10 | 17.1 | 4 | 5 | 5.74 |
| | 10 | 15.7 | 6 | 5 | 6.26 |
| | 10 | 17.8 | 4 | 5 | 5.48 |
| | 30 | 17.1 | 6 | 5 | 5.6 |
| | 30 | 15.7 | 8 | 5 | 5.97 |
| | 30 | 14.2 | 8 | 5 | 5.2 |
| | 60 | 14.6 | 8 | 5 | 5.47 |
| | 60 | 14.1 | 9 | 5 | 5.47 |
| | 60 | 13 | 9 | 5 | 5.56 |
| Crowley | 0 | 17.9 | 5 | 2 | 3.18 |
| | 0 | 18.4 | 5 | 3 | 3.36 |
| | 0 | 17 | 4 | 4 | 4.09 |
| | 10 | 16.4 | 6 | 4 | 3.77 |
| | 10 | 16 | 6 | 5 | 3.72 |
| | 10 | 14.7 | 5 | 5 | 4.25 |
| | 30 | 13 | 9 | 5 | 4.15 |
| | 30 | 12.5 | 8 | 5 | 4.22 |
| | 30 | 13 | 7 | 5 | 4.15 |
| | 60 | 12.1 | 9 | 5 | 4.89 |
| | 60 | 11.9 | 8 | 5 | 5.11 |
| | 60 | 12.4 | 8 | 5 | 5.05 |
| Daisy I | 0 | 16.3 | 6 | 5 | 6.53 |
| | 0 | 16.9 | 6 | 5 | 5.77 |
| | 0 | 19.4 | 5 | 4 | 5.65 |
| | 10 | 16.1 | 7 | 5 | 5.24 |
| | 10 | 16.1 | 7 | 5 | 4.75 |
| | 10 | 18.1 | 6 | 5 | 4.86 |
| | 30 | 16.5 | 7 | 5 | 4.56 |
| | 30 | 16.4 | 7 | 5 | 4.49 |
| | 30 | 16.5 | 7 | 5 | 4.5 |
| Lake Laurentian | 0 | 17.9 | 6 | 3 | 4.4 |
| | 0 | 21.4 | 6 | 4 | 4.42 |
| | 0 | 19.4 | 5 | 3 | 4.29 |
| | 10 | 14.9 | 6 | 5 | 4.58 |
| | 10 | 16.4 | 6 | 5 | 4.69 |
| | 10 | 16.3 | 6 | 4 | 4.52 |
| | 30 | 14.2 | 9 | 5 | 4.37 |
| | 30 | 16.5 | 8 | 5 | 5.03 |
| | 30 | 16.2 | 8 | 5 | 5.1 |
| | 60 | 13.8 | 6 | 5 | 5.1 |
| | 60 | 15.2 | 8 | 5 | 5.6 |
| | 60 | 14.3 | 8 | 5 | 5.51 |
| Clearwater | 0 | 15.1 | 4 | 4 | 3.88 |
| | 0 | 14.2 | 4 | 4 | 3.65 |
| | 0 | 13 | 4 | 5 | 3.7 |
| | 10 | 13 | 5 | 5 | 3.73 |
| | 10 | 14.9 | 5 | 4 | 3.76 |
| | 10 | 13 | 5 | 5 | 3.84 |
| | 30 | 13 | 7 | 5 | 3.78 |
| | 30 | 13.4 | 9 | 5 | 3.61 |
| | 30 | 13 | 7 | 5 | 3.67 |
| | 60 | 12.6 | 7 | 5 | 3.94 |
| | 60 | 12.2 | 6 | 5 | 3.68 |
| | 60 | 12 | 7 | 5 | 3.67 |
| Rockcut | 0 | 13.7 | 3 | 4 | 3.92 |
| | 0 | 13.1 | 3 | 4 | 4.31 |
| | 0 | 17.3 | 4 | 4 | 4.06 |
| | 10 | 13.5 | 4 | 5 | 4.51 |
| | 10 | 13.3 | 5 | 5 | 4.33 |
| | 10 | 13.1 | 5 | 5 | 4.04 |
| | 30 | 14.6 | 6 | 5 | 4.23 |
| | 30 | 14.2 | 8 | 5 | 4.14 |
| | 30 | 14.9 | 7 | 5 | 4.35 |
| | 60 | 14.5 NA | | 5 | 4.5 |
| | 60 | 13.8 NA | | 5 | 4.7 |
| | 60 | 13.8 | 9 | 5 | 4.65 |
| Whitson | 0 | 22.4 | 4 | 4 | 7.44 |
| | 0 | 17.1 | 3 | 4 | 7.53 |
| | 0 | 13 | 3 | 4 | 6.44 |
| | 10 | 14.1 | 5 | 5 | 7.07 |
| | 10 | 13.3 | 4 | 5 | 6.26 |
| | 10 | 13 | 5 | 5 | 5.81 |
| | 30 | 14.7 | 6 | 5 | 5.61 |
| | 30 | 15 | 8 | 5 | 5.24 |
| | 30 | 14.5 | 7 | 5 | 5.61 |
| | 60 | 14 NA | | 5 | 5.93 |
| | 60 | 13.1 NA | | 5 | 5.64 |
| | 60 | 13.2 NA | | 5 | 5.69 |

## 7.4 Peat Metal Analysis

**Table 7.21-** Peat metal concentration (mg/kg) for the low-impacted sites along the Sudbury metal gradient.

| Peat | Sites | Ag | Al | As | B | Ba | Be | Bi | Ca | Cd | Co | Cr | Cu | Fe | Hg | In | K | Mg | Mn | Mo | Na | Ni | P | Pb | Rb | Sb | Sc | Se | Sn | Sr | Th | Ti | Tl | U | V | W | Y | Zn | Zr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CONTROL A | | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 0.1 | <DL | <DL | <DL | 0.4 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 0.1 | <DL |
| CONTROL B | | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 0.1 | <DL |
| CA1P1S1 | Cartier 1 | <DL | 6.7 | <DL | 1.8 | 3.8 | <DL | <DL | 212 | <DL | 0.1 | <DL | 0.1 | 33 | <DL | <DL | 803 | 137 | 4.3 | <DL | 202 | 0.6 | 6.7 | <DL | 3.5 | <DL | <DL | <DL | <DL | 1.5 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 10 | <DL |
| CA1P1S2 | | <DL | 8.8 | 0.2 | 2.9 | 13 | <DL | <DL | 333 | <DL | 0.2 | <DL | 0.2 | 54 | <DL | <DL | 584 | 164 | 5.6 | <DL | 386 | 1.1 | 17 | <DL | 4.8 | <DL | <DL | <DL | <DL | 2.5 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 14 | <DL |
| CA1P1S3 | | <DL | 17 | 0.2 | 2 | 5.9 | <DL | <DL | 446 | <DL | 0.2 | <DL | 0.3 | 80 | <DL | <DL | 109 | 193 | 4 | <DL | 444 | 1.6 | <DL | <DL | 1 | <DL | <DL | <DL | <DL | 3.2 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 12 | <DL |
| CA1P1S4 | | <DL | 8.5 | <DL | 2.5 | 11 | <DL | <DL | 291 | <DL | <DL | <DL | <DL | 38 | <DL | <DL | 15 | 118 | 0.5 | <DL | 226 | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | 2.4 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 9.1 | <DL |
| CA1P2S1 | | <DL | 1.8 | <DL | 1.9 | 4.6 | <DL | <DL | 186 | <DL | 0 | <DL | 0.1 | 21 | <DL | <DL | 640 | 131 | 14 | <DL | 205 | 0.4 | <DL | <DL | 3.1 | <DL | <DL | <DL | <DL | 0.8 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 9 | <DL |
| CA1P2S2 | | <DL | 5 | <DL | 2.7 | 7.6 | <DL | <DL | 266 | <DL | 0.1 | <DL | 0.1 | 35 | <DL | <DL | 648 | 175 | 9 | <DL | 238 | 0.9 | <DL | <DL | 3.3 | <DL | <DL | <DL | <DL | 1.5 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 15 | <DL |
| CA1P2S3 | | <DL | 14 | 0.3 | 2.3 | 6.5 | <DL | <DL | 457 | <DL | 0.1 | <DL | <DL | 71 | <DL | <DL | 48 | 199 | 3.6 | <DL | 137 | 1.2 | <DL | <DL | 0.1 | <DL | <DL | <DL | <DL | 3.2 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 12 | <DL |
| CA1P2S4 | | <DL | 8.4 | <DL | 1.4 | 3.9 | <DL | <DL | 278 | <DL | 0 | <DL | 0.1 | 39 | <DL | <DL | 9.7 | 129 | 0.9 | <DL | 94 | 0.1 | <DL | <DL | 0 | <DL | <DL | <DL | <DL | 2.1 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 5.7 | <DL |
| CA1P3S1 | | <DL | 6.5 | <DL | 3.1 | 13 | <DL | <DL | 231 | 0.1 | 0.1 | <DL | 0.3 | 35 | <DL | <DL | 520 | 166 | 3.2 | <DL | 222 | 1.1 | <DL | <DL | 3.5 | <DL | <DL | <DL | <DL | 1.9 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 19 | <DL |
| CA1P3S2 | | <DL | 13 | 0.1 | 1.9 | 5.9 | <DL | <DL | 348 | 0.1 | 0.2 | <DL | 0.1 | 61 | <DL | <DL | 203 | 183 | 2.4 | <DL | 142 | 1.5 | 52 | <DL | 1.5 | <DL | <DL | <DL | <DL | 2.5 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 15 | <DL |
| CA1P3S3 | | <DL | 9.3 | <DL | 2.6 | 11 | <DL | <DL | 341 | <DL | 0 | <DL | <DL | 52 | <DL | <DL | 16 | 133 | 0.6 | <DL | 127 | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | 2.9 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 10 | <DL |
| CA1P3S4 | | <DL | 12 | <DL | 3.1 | 14 | <DL | <DL | 369 | <DL | 0 | <DL | <DL | 52 | <DL | <DL | 9.5 | 138 | 0.2 | <DL | 153 | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | 3.3 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 11 | <DL |
| CA2P1S1 | Cartier 2 | <DL | 7.3 | <DL | 3.2 | 5 | <DL | <DL | 226 | <DL | 0.1 | <DL | 0.1 | 30 | <DL | <DL | 237 | 120 | 3.2 | <DL | 469 | 0.9 | <DL | <DL | 2 | <DL | <DL | <DL | <DL | 1.9 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 11 | <DL |
| CA2P1S2 | | <DL | 11 | 0.1 | 5.2 | 4.5 | <DL | <DL | 257 | <DL | 0.1 | <DL | 0.2 | 36 | <DL | <DL | 99 | 132 | 2 | <DL | 474 | 1.2 | <DL | <DL | 0.9 | <DL | <DL | <DL | <DL | 2.3 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 11 | <DL |
| CA2P1S3 | | <DL | 15 | <DL | 4.5 | 18 | <DL | <DL | 481 | <DL | 0.1 | <DL | <DL | 86 | <DL | <DL | 34 | 206 | 1.5 | <DL | 277 | 1.3 | <DL | <DL | 0 | <DL | <DL | <DL | <DL | 4.2 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 21 | <DL |
| CA2P1S4 | | <DL | 15 | <DL | 3.9 | 8.9 | <DL | <DL | 455 | <DL | <DL | <DL | 0.2 | 69 | <DL | <DL | 26 | 161 | 0.4 | <DL | 328 | 0.1 | <DL | <DL | 0.1 | <DL | <DL | <DL | <DL | 3.9 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 13 | <DL |
| CA2P2S1 | | <DL | 10 | <DL | 5 | 9.5 | <DL | <DL | 373 | <DL | 0.2 | <DL | 0.2 | 48 | <DL | <DL | 48 | 149 | 2 | <DL | 293 | 2.1 | <DL | <DL | 0.2 | <DL | <DL | <DL | <DL | 2.9 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 22 | <DL |
| CA2P2S2 | | <DL | 13 | 0.1 | 1.9 | 6.2 | <DL | <DL | 384 | <DL | 0.2 | <DL | 0.2 | 53 | <DL | <DL | 47 | 161 | 2.4 | <DL | 196 | 2.5 | <DL | <DL | 0.4 | <DL | <DL | <DL | <DL | 3 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 16 | <DL |
| CA2P2S3 | | <DL | 17 | <DL | 4 | 10 | <DL | <DL | 483 | <DL | 0.1 | <DL | 0.2 | 83 | <DL | <DL | 75 | 183 | 1.4 | <DL | 216 | 1.4 | <DL | <DL | 0.4 | <DL | <DL | <DL | <DL | 3.8 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 17 | <DL |
| CA2P2S4 | | <DL | 19 | <DL | 2.7 | 6.4 | <DL | <DL | 435 | <DL | 0 | <DL | 0 | 66 | <DL | <DL | 11 | 154 | 0.5 | <DL | 171 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 3.2 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 11 | <DL |
| CA2P3S1 | | <DL | 9.9 | <DL | 8 | 9.1 | <DL | <DL | 386 | <DL | 0.2 | <DL | 0.1 | 49 | <DL | <DL | 220 | 193 | 5.7 | <DL | 291 | 1.7 | <DL | <DL | 1.2 | <DL | 0.3 | <DL | <DL | 2.5 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 21 | <DL |
| CA2P3S2 | | <DL | 12 | <DL | 3.7 | 10 | <DL | <DL | 372 | <DL | 0.1 | <DL | 0.1 | 52 | <DL | <DL | 135 | 155 | 2 | <DL | 189 | 1.9 | <DL | <DL | 0.8 | <DL | <DL | <DL | <DL | 2.7 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 17 | <DL |
| CA2P3S3 | | <DL | 14 | <DL | 4.4 | 9.8 | <DL | <DL | 505 | <DL | 0 | <DL | 0.2 | 83 | <DL | <DL | 23 | 166 | 0.8 | <DL | 211 | 0.4 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 4.1 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 16 | <DL |
| CA2P3S4 | | <DL | 18 | <DL | 4 | 10 | <DL | <DL | 539 | <DL | 0 | <DL | 0.5 | 84 | <DL | <DL | 4.5 | 177 | 0.4 | <DL | 249 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 4.1 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 15 | <DL |

**Table 7.22-** Peat metal concentration (mg/kg) for the intermediate-low impacted sites along the Sudbury metal gradient.

| Peat | Sites | Ag | Al | As | B | Ba | Be | Bi | Ca | Cd | Co | Cr | Cu | Fe | Hg | In | K | Mg | Mn | Mo | Na | Ni | P | Pb | Rb | Sb | Sc | Se | Sn | Sr | Th | Ti | Tl | U | V | W | Y | Zn | Zr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CONTROL A | | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 0.1 | <DL | <DL | <DL | 0.4 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 0.1 | <DL |
| CONTROL B | | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL |
| ASP1S1 | Ashigami | <DL | 4.2 | 0.4 | 2.4 | 11 | <DL | <DL | 488 | <DL | 0.6 | <DL | 0.2 | 70 | <DL | <DL | 264 | 124 | 19 | <DL | 151 | 4.2 | <DL | <DL | 1.4 | <DL | <DL | <DL | <DL | 2.1 | <DL | <DL | <DL | <DL | <DL | <DL | 10 | <DL |
| ASP1S2 | | <DL | 6.6 | 2.7 | 2.3 | 8.7 | <DL | <DL | 544 | <DL | 0.7 | <DL | 0.2 | 74 | <DL | <DL | 149 | 121 | 21 | <DL | 115 | 4.6 | <DL | <DL | 1 | <DL | <DL | <DL | <DL | 2.3 | <DL | <DL | <DL | <DL | <DL | <DL | 11 | <DL |
| ASP1S3 | | <DL | 13 | 11 | 2.6 | 7.1 | <DL | <DL | 828 | <DL | 0.7 | <DL | 0.3 | 119 | <DL | <DL | 29 | 169 | 33 | <DL | 85 | 5 | <DL | <DL | 0.1 | <DL | <DL | <DL | <DL | 3.5 | <DL | <DL | <DL | <DL | <DL | <DL | 11 | <DL |
| ASP1S4 | | <DL | 5.8 | 0.2 | 1.7 | 4.4 | <DL | <DL | 619 | <DL | 0 | <DL | 0.1 | 89 | <DL | <DL | 31 | 166 | 2.8 | <DL | 75 | 0.1 | <DL | <DL | 0 | <DL | 0.1 | <DL | <DL | 2.4 | <DL | <DL | <DL | <DL | <DL | <DL | 4.6 | <DL |
| ASP2S1 | | <DL | 15 | 0.2 | 1.2 | 2.4 | <DL | <DL | 180 | <DL | 0.5 | <DL | 0.9 | 42 | <DL | <DL | 529 | 58 | 5.7 | <DL | 217 | 4.7 | <DL | <DL | 1.8 | <DL | <DL | <DL | <DL | 0.6 | <DL | <DL | <DL | <DL | <DL | <DL | 5.6 | <DL |
| ASP2S2 | | <DL | 6.1 | 0.7 | 2.1 | 9.1 | <DL | <DL | 239 | <DL | 0.4 | <DL | 0.7 | 47 | <DL | <DL | 511 | 75 | 11 | <DL | 214 | 3 | <DL | <DL | 2.1 | <DL | <DL | 0.2 | <DL | 1.1 | <DL | 0.4 | <DL | <DL | <DL | <DL | 7.4 | <DL |
| ASP2S3 | | <DL | 4.7 | 8.8 | 2.9 | 7.6 | <DL | <DL | 870 | <DL | 0.4 | <DL | 0.2 | 131 | <DL | <DL | 24 | 182 | 22 | <DL | 86 | 3.1 | <DL | <DL | 0.1 | <DL | 0.1 | <DL | <DL | 3.4 | <DL | 0.4 | <DL | <DL | <DL | <DL | 8.5 | <DL |
| ASP2S4 | | <DL | 5.4 | 0.2 | 2.1 | 9.2 | <DL | <DL | 641 | <DL | 0 | <DL | 0 | 92 | <DL | <DL | 23 | 157 | 2.5 | <DL | 72 | 0.2 | <DL | <DL | 0.1 | <DL | 0.1 | <DL | <DL | 2.7 | <DL | <DL | <DL | <DL | <DL | <DL | 5.3 | <DL |
| ASP3S1 | | <DL | 7.6 | 1.4 | 1.2 | 3.8 | <DL | <DL | 266 | <DL | 0.5 | <DL | 0.3 | 39 | <DL | <DL | 354 | 85 | 10 | <DL | 105 | 3.5 | <DL | <DL | 2.1 | <DL | <DL | <DL | <DL | 1 | <DL | <DL | <DL | <DL | <DL | <DL | 6.5 | <DL |
| ASP3S2 | | <DL | 11 | 3.2 | 2 | 7.4 | <DL | <DL | 498 | 0 | 0.9 | <DL | 0.4 | 71 | <DL | <DL | 184 | 123 | 18 | <DL | 210 | 6.8 | <DL | <DL | 0.8 | <DL | <DL | <DL | <DL | 2 | <DL | <DL | <DL | <DL | <DL | <DL | 11 | <DL |
| ASP3S3 | | <DL | 3.9 | 3.6 | 3.5 | 9.5 | <DL | <DL | 809 | <DL | 0.3 | <DL | 0.1 | 119 | <DL | <DL | 29 | 157 | 15 | <DL | 104 | 1.6 | <DL | <DL | 0.1 | <DL | 0.1 | <DL | <DL | 3.3 | <DL | 0.3 | <DL | <DL | <DL | <DL | 6.9 | <DL |
| ASP3S4 | | <DL | 5.5 | 0.2 | 1.9 | 6.6 | <DL | <DL | 655 | <DL | 0 | <DL | 0 | 93 | <DL | <DL | 17 | 166 | 2.5 | <DL | 81 | 0.2 | <DL | <DL | 0 | <DL | 0.1 | <DL | <DL | 2.7 | <DL | <DL | <DL | <DL | <DL | <DL | 5.6 | <DL |
| BRP1S1 | Broder | <DL | 39 | 0.6 | 1.8 | 9.4 | <DL | <DL | 301 | <DL | 0.8 | <DL | 1.8 | 45 | <DL | <DL | 628 | 84 | 10 | <DL | 115 | 14 | <DL | <DL | 2.4 | <DL | <DL | <DL | <DL | 1.7 | <DL | <DL | <DL | <DL | <DL | <DL | 8.5 | <DL |
| BRP1S2 | | <DL | 36 | 0.4 | 2.1 | 7.7 | <DL | <DL | 273 | <DL | 1.2 | <DL | 1.1 | 36 | <DL | <DL | 253 | 88 | 13 | <DL | 134 | 15 | <DL | <DL | 0.9 | <DL | <DL | <DL | <DL | 1.8 | <DL | <DL | <DL | <DL | <DL | <DL | 8.8 | <DL |
| BRP1S3 | | <DL | 9.1 | 0.1 | 1.2 | 4.2 | <DL | <DL | 591 | <DL | 0.1 | <DL | 0 | 83 | <DL | <DL | 7.2 | 152 | 4.1 | <DL | 64 | 0.6 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 2.9 | <DL | <DL | <DL | <DL | <DL | <DL | 4.2 | <DL |
| BRP1S4 | | <DL | 7.9 | 0.1 | 1.6 | 7.9 | <DL | <DL | 622 | <DL | 0 | <DL | 0 | 89 | <DL | <DL | 12 | 174 | 1.5 | <DL | 74 | 0.1 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 2.9 | <DL | <DL | <DL | <DL | 0.1 | <DL | <DL | 5.6 | <DL |
| BRP2S1 | | <DL | 32 | 1.2 | 2.5 | 7.9 | <DL | <DL | 241 | <DL | 0.7 | <DL | 1.8 | 31 | <DL | <DL | 696 | 69 | 6.6 | <DL | 210 | 11 | <DL | <DL | 2.7 | <DL | <DL | <DL | <DL | 1.5 | <DL | <DL | <DL | <DL | <DL | <DL | 9.7 | <DL |
| BRP2S2 | | <DL | 33 | 2.6 | 4.3 | 9.6 | <DL | <DL | 347 | 0.1 | 1.1 | <DL | 2.3 | 57 | <DL | <DL | 150 | 76 | 9.7 | <DL | 118 | 23 | <DL | <DL | 0.4 | <DL | 0.2 | <DL | <DL | 2.4 | <DL | <DL | <DL | <DL | <DL | <DL | 12 | <DL |
| BRP2S3 | | <DL | 7.8 | 0.5 | 2 | 5.8 | <DL | <DL | 662 | <DL | 0.2 | <DL | 0.1 | 95 | <DL | <DL | 16 | 124 | 9.8 | <DL | 80 | 1.9 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 3.6 | <DL | <DL | <DL | <DL | <DL | <DL | 5.6 | <DL |
| BRP2S4 | | <DL | 7 | <DL | 1 | 3.6 | <DL | <DL | 623 | <DL | 0 | <DL | 0 | 89 | <DL | <DL | 5.2 | 176 | 2.3 | <DL | 58 | 0.1 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 2.5 | <DL | <DL | <DL | <DL | 0.1 | <DL | 3.2 | <DL |
| BRP3S1 | | <DL | 21 | 0.4 | 1.8 | 11 | <DL | <DL | 216 | <DL | 0.8 | <DL | 1.4 | 27 | <DL | <DL | 409 | 78 | 8.9 | <DL | 179 | 10 | <DL | <DL | 1.4 | <DL | 0.1 | <DL | <DL | 1.4 | <DL | <DL | <DL | <DL | <DL | <DL | 9.9 | <DL |
| BRP3S2 | | <DL | 17 | 1.7 | 1.4 | 7 | <DL | <DL | 254 | 0 | 0.9 | <DL | 1.6 | 33 | <DL | <DL | 115 | 68 | 8 | <DL | 109 | 16 | <DL | <DL | 0.2 | <DL | 0.1 | <DL | <DL | 1.7 | <DL | <DL | <DL | <DL | <DL | <DL | 7 | <DL |
| BRP3S3 | | <DL | 8.1 | 0.2 | 1.9 | 9.6 | <DL | <DL | 630 | <DL | 0.1 | <DL | 0 | 91 | <DL | <DL | 15 | 103 | 7.5 | <DL | 79 | 0.4 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 3.3 | <DL | <DL | <DL | <DL | <DL | <DL | 6 | <DL |
| BRP3S4 | | <DL | 5.7 | <DL | 1.4 | 3.7 | <DL | <DL | 620 | <DL | 0 | <DL | 0.1 | 90 | <DL | <DL | 7 | 143 | 2.8 | <DL | 71 | 0.1 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 2.4 | <DL | <DL | <DL | <DL | 0.1 | <DL | <DL | 4.7 | <DL |
| ROP1S1 | Rockcut | <DL | 128 | 0.8 | 3.3 | 12 | <DL | <DL | 190 | 0.1 | 1.5 | <DL | 1.5 | 108 | <DL | <DL | 380 | 84 | 9 | <DL | 137 | 13 | <DL | <DL | 2.5 | <DL | 0.2 | <DL | <DL | 1.7 | <DL | <DL | <DL | <DL | <DL | <DL | 13 | <DL |
| ROP1S2 | | <DL | 40 | 2.1 | 5.1 | 5.3 | <DL | <DL | 144 | <DL | 0.3 | <DL | 0.8 | 24 | <DL | <DL | 175 | 52 | 4 | <DL | 127 | 3.3 | <DL | <DL | 0.6 | <DL | 0.5 | 0.3 | <DL | 1 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 9 | <DL |
| ROP1S3 | | <DL | 24 | 1 | 3 | 5.3 | <DL | <DL | 229 | <DL | 0.3 | <DL | 0.2 | 35 | <DL | <DL | 64 | 61 | 7.3 | <DL | 73 | 3.7 | <DL | <DL | 0.1 | <DL | 0.3 | <DL | <DL | 1.8 | <DL | 0.7 | <DL | <DL | <DL | <DL | 7.4 | <DL |
| ROP1S4 | | <DL | 10 | 0.1 | 1 | 1.1 | <DL | <DL | 84 | <DL | 0 | <DL | 0 | 12 | <DL | <DL | 6.4 | 13 | 2.4 | <DL | 22 | 0.2 | <DL | <DL | <DL | <DL | 0.2 | 0.1 | <DL | 0.6 | <DL | 0.2 | <DL | <DL | 0 | <DL | <DL | 1.8 | <DL |
| ROP2S1 | | <DL | 104 | 0.7 | 2.2 | 5.3 | <DL | <DL | 103 | 0 | 0.8 | <DL | 0.9 | 43 | <DL | <DL | 341 | 55 | 6.2 | <DL | 89 | 5.4 | <DL | <DL | 1.7 | <DL | 0.2 | <DL | <DL | 0.7 | <DL | <DL | <DL | <DL | <DL | <DL | 8.4 | <DL |
| ROP2S2 | | <DL | 35 | 3.3 | 1.8 | 3.2 | <DL | <DL | 68 | <DL | 0.1 | <DL | 0.8 | 9.3 | <DL | <DL | 148 | 32 | 2.3 | <DL | 76 | 1.6 | <DL | <DL | 0.4 | <DL | 0.2 | <DL | <DL | 0.4 | <DL | 0.3 | <DL | <DL | <DL | <DL | 5 | <DL |
| ROP2S3 | | <DL | 25 | 0.8 | 2.1 | 6.6 | <DL | <DL | 223 | <DL | 0.4 | <DL | 0.1 | 32 | <DL | <DL | 111 | 74 | 8.1 | <DL | 79 | 4.5 | <DL | <DL | 0.1 | <DL | 0.2 | <DL | <DL | 1.7 | <DL | <DL | <DL | <DL | <DL | <DL | 7.5 | <DL |
| ROP2S4 | | <DL | 9.1 | <DL | 0.5 | 1.1 | <DL | <DL | 64 | <DL | 0 | <DL | 0 | 8.7 | <DL | <DL | 3.4 | 10 | 1.7 | <DL | 17 | 0.1 | <DL | <DL | <DL | <DL | 0.1 | <DL | <DL | 0.4 | <DL | <DL | <DL | <DL | 0 | <DL | <DL | 1.1 | <DL |
| ROP3S1 | | <DL | 96 | 0.9 | 1.9 | 4.7 | <DL | <DL | 87 | <DL | 0.7 | <DL | 0.7 | 88 | <DL | <DL | 200 | 49 | 7.6 | <DL | 89 | 5.6 | <DL | <DL | 0.4 | <DL | 0.1 | <DL | <DL | 0.6 | <DL | <DL | <DL | <DL | <DL | <DL | 6 | <DL |
| ROP3S2 | | <DL | 93 | 0.8 | 3.4 | 3.7 | <DL | <DL | 100 | <DL | 0.6 | <DL | 0.6 | 97 | <DL | <DL | 236 | 57 | 10 | <DL | 74 | 6 | <DL | <DL | 0.6 | <DL | 0.2 | <DL | <DL | 0.7 | <DL | <DL | <DL | <DL | <DL | <DL | 8.1 | <DL |
| ROP3S3 | | <DL | 21 | 0.4 | 3.9 | 5.2 | <DL | <DL | 320 | <DL | 0.3 | <DL | 0 | 50 | <DL | <DL | 83 | 91 | 10 | <DL | 89 | 2.8 | <DL | <DL | 0.1 | <DL | 0.4 | <DL | <DL | 2.4 | <DL | <DL | <DL | <DL | <DL | <DL | 7.8 | <DL |
| ROP3S4 | | <DL | 9.7 | <DL | 1.6 | 3.4 | <DL | <DL | 232 | <DL | 0 | <DL | 0 | 34 | <DL | <DL | 17 | 90 | 2.2 | <DL | 51 | 0.1 | <DL | <DL | <DL | <DL | 0.3 | <DL | <DL | 1.6 | <DL | 0.3 | <DL | <DL | <DL | <DL | 3.8 | <DL |

**Table 7.23-** Peat metal concentration (mg/kg) for the intermediate-high impacted sites along the Sudbury metal gradient.

| Peat | Sites | Ag | Al | As | B | Ba | Be | Bi | Ca | Cd | Co | Cr | Cu | Fe | Hg | In | K | Mg | Mn | Mo | Na | Ni | P | Pb | Rb | Sb | Sc | Se | Sn | Sr | Th | Ti | Tl | U | V | W | Y | Zn | Zr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CONTROL A | | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 0.14 | <DL | <DL | <DL | 0.4 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 0.1 | <DL |
| CONTROL B | | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 0.1 | <DL |
| CRP1S1 | Crowley | <DL | 6.8 | 0.2 | 1.1 | 3.1 | <DL | <DL | 184 | 0.1 | 0.4 | 0.1 | 3.4 | 31 | <DL | <DL | 317 | 72 | 3.2 | <DL | 71 | 7.5 | 79 | 0 | 1.9 | <DL | 0.1 | <DL | <DL | 0.8 | <DL | 0.2 | <DL | <DL | 0 | <DL | <DL | 5.1 | <DL |
| CRP1S2 | | <DL | 22 | 4.7 | 1.5 | 4.9 | <DL | <DL | 287 | 0 | 0.7 | <DL | 6 | 162 | <DL | <DL | 285 | 87 | 4.9 | <DL | 116 | 16 | <DL | 0.2 | 1.7 | <DL | <DL | 0.2 | <DL | 1.7 | <DL | 0.3 | <DL | <DL | 0.1 | <DL | <DL | 7.3 | <DL |
| CRP1S3 | | <DL | 13 | 1.1 | 2.1 | 4.5 | <DL | <DL | 512 | <DL | 0.1 | <DL | 0.2 | 73 | <DL | <DL | 4.85 | 144 | 2.4 | <DL | 89 | 1.4 | <DL | <DL | <DL | <DL | 0.1 | <DL | <DL | 3 | <DL | 0.5 | <DL | <DL | <DL | <DL | <DL | 6.1 | <DL |
| CRP1S4 | | <DL | 7.9 | 0.4 | 2.5 | 4.9 | <DL | <DL | 575 | <DL | <DL | <DL | 0 | 88 | <DL | <DL | 17.7 | 258 | 0.8 | <DL | 87 | 0.1 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 2.8 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 5.7 | <DL |
| CRP2S1 | | <DL | 19 | 0.4 | 2 | 3.9 | <DL | <DL | 239 | 0.1 | 0.6 | <DL | 6.6 | 116 | <DL | <DL | 314 | 100 | 3.4 | <DL | 82 | 14 | <DL | 0.1 | 1.4 | <DL | 0.1 | <DL | <DL | 1.2 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 8 | <DL |
| CRP2S2 | | <DL | 21 | 3.6 | 2.2 | 6 | <DL | <DL | 456 | 0 | 0.8 | <DL | 4.6 | 108 | <DL | <DL | 146 | 141 | 2.4 | <DL | 77 | 19 | <DL | 0 | 0.6 | <DL | 0.1 | <DL | <DL | 2.9 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 8.5 | <DL |
| CRP2S3 | | <DL | 6.7 | 1.8 | 3 | 4.8 | <DL | <DL | 581 | <DL | 0.2 | <DL | 0.2 | 92 | <DL | <DL | 25.7 | 180 | 3 | <DL | 84 | 3 | <DL | <DL | 0 | <DL | 0.2 | <DL | <DL | 3.4 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 7.4 | <DL |
| CRP2S4 | | <DL | 8.2 | 0.1 | 2.3 | 4.3 | <DL | <DL | 600 | <DL | 0 | <DL | 0.1 | 93 | <DL | <DL | 22.1 | 277 | 0.9 | <DL | 89 | 0.2 | <DL | <DL | 0 | <DL | 0.2 | <DL | <DL | 2.8 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 5.4 | <DL |
| CRP3S1 | | <DL | 25 | 2.6 | 2.6 | 6.2 | <DL | <DL | 471 | 0 | 0.9 | <DL | 4.1 | 133 | <DL | <DL | 869 | 152 | 4.4 | <DL | 124 | 16 | 57 | 0 | 4.5 | <DL | 0.4 | <DL | <DL | 3 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 9.4 | <DL |
| CRP3S2 | | <DL | 21 | 4.9 | 2.9 | 7.2 | <DL | <DL | 575 | 0 | 0.8 | <DL | 2 | 113 | <DL | <DL | 418 | 191 | 5.3 | <DL | 106 | 15 | 80 | 0.1 | 2.5 | <DL | 0.2 | <DL | <DL | 3.6 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 9.2 | <DL |
| CRP3S3 | | <DL | 6.6 | 1.8 | 4.4 | 3.1 | <DL | <DL | 570 | <DL | 0.1 | <DL | 0.1 | 87 | <DL | <DL | 15.8 | 189 | 2.3 | <DL | 91 | 0.6 | <DL | <DL | <DL | <DL | 0.3 | <DL | <DL | 3.2 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 6.9 | <DL |
| CRP3S4 | | <DL | 8 | <DL | 4.1 | 2.9 | <DL | <DL | 613 | <DL | <DL | <DL | 0.1 | 94 | <DL | <DL | 21 | 265 | 0.8 | <DL | 94 | 0.2 | <DL | <DL | 0 | <DL | 0.3 | <DL | <DL | 3 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 6.8 | <DL |
| DAP1S1 | Daisy I | <DL | 34 | 0.3 | 3.4 | 2.7 | <DL | <DL | 53 | <DL | 0.1 | <DL | 4.8 | 38 | <DL | <DL | 813 | 14 | 1.5 | <DL | 148 | 1.7 | <DL | <DL | 2.9 | <DL | 0.6 | <DL | <DL | 0.1 | <DL | 0.4 | <DL | <DL | <DL | <DL | <DL | 4.8 | <DL |
| DAP1S2 | | <DL | 17 | 0.8 | 2.3 | 2.2 | <DL | <DL | 76 | <DL | 0.3 | <DL | 3 | 34 | <DL | <DL | 321 | 44 | 3.1 | <DL | 106 | 3.6 | <DL | <DL | 1 | <DL | 0.6 | <DL | <DL | 0.3 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 5.3 | <DL |
| DAP1S3 | | <DL | 7 | <DL | 0.9 | 10 | <DL | <DL | 210 | 0 | 0.6 | <DL | 0.1 | 30 | <DL | <DL | 18.9 | 64 | 6.6 | <DL | 43 | 15 | <DL | <DL | 0 | <DL | 0.4 | <DL | <DL | 1.4 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 4.1 | <DL |
| DAP2S1 | | <DL | 39 | 0.6 | 5.2 | 1.7 | <DL | <DL | 56 | <DL | 0.1 | <DL | 4.1 | 34 | <DL | <DL | 518 | 14 | 0.9 | <DL | 131 | 1.4 | <DL | <DL | 1.7 | <DL | 0.7 | <DL | <DL | 0.1 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 7.1 | <DL |
| DAP2S2 | | <DL | 20 | 1.6 | 1.6 | 6.3 | <DL | <DL | 115 | 0 | 0.7 | <DL | 1.9 | 32 | <DL | <DL | 95.8 | 49 | 5 | <DL | 77 | 9.7 | <DL | <DL | 0.4 | <DL | 0.5 | <DL | <DL | 0.5 | <DL | 0.3 | <DL | <DL | <DL | <DL | <DL | 5.3 | <DL |
| DAP2S3 | | <DL | 14 | 0.1 | 0.7 | 7.1 | <DL | <DL | 116 | 0 | 0.6 | <DL | 0.7 | 25 | <DL | <DL | 9.93 | 43 | 5.5 | <DL | 39 | 9.5 | <DL | <DL | 0 | <DL | 0.3 | <DL | <DL | 0.8 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 3.1 | <DL |
| DAP3S1 | | <DL | 66 | 0.5 | 2.2 | 3.2 | <DL | <DL | 52 | <DL | 0.1 | <DL | 5.1 | 95 | <DL | <DL | 656 | 23 | 2.2 | <DL | 95 | 2 | <DL | <DL | 1.8 | <DL | 0.6 | <DL | <DL | 0.1 | <DL | 0.4 | <DL | <DL | <DL | <DL | 0 | 5.6 | <DL |
| DAP3S2 | | <DL | 29 | 2.7 | 1.9 | 4.8 | <DL | <DL | 99 | <DL | 0.5 | <DL | 2.1 | 27 | <DL | <DL | 114 | 43 | 3.9 | <DL | 96 | 6.2 | <DL | <DL | 0.3 | <DL | 0.4 | <DL | <DL | 0.5 | <DL | 0.4 | <DL | <DL | <DL | <DL | <DL | 4.9 | <DL |
| DAP3S3 | | <DL | 11 | 0.2 | 1 | 6.8 | <DL | <DL | 178 | 0 | 0.7 | <DL | 0.3 | 25 | <DL | <DL | 11.9 | 62 | 6.8 | <DL | 49 | 9.8 | <DL | <DL | 0 | <DL | 0.3 | <DL | <DL | 1.2 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 3.8 | <DL |
| LOP1S1 | Long Lake | <DL | 25 | 5.3 | 4.1 | 5.8 | <DL | <DL | 349 | <DL | 0.7 | <DL | 4.7 | 195 | <DL | <DL | 1030 | 95 | 4.4 | <DL | 143 | 13 | <DL | 0.1 | 4.7 | <DL | 0.2 | <DL | <DL | 1.5 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 14 | <DL |
| LOP1S2 | | <DL | 27 | 4.2 | 3.1 | 7.2 | <DL | <DL | 680 | <DL | 0.7 | <DL | 8.9 | 114 | <DL | <DL | 84.9 | 106 | 4.5 | <DL | 196 | 16 | <DL | 0 | 0.3 | <DL | 0.2 | <DL | <DL | 3 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 12 | <DL |
| LOP1S3 | | <DL | 18 | 0.1 | 2.2 | 3.2 | <DL | <DL | 321 | <DL | 0 | <DL | 0.1 | 49 | <DL | <DL | 20.1 | 127 | 2.7 | <DL | 112 | 0.2 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 1.3 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 5.2 | <DL |
| LOP1S4 | | <DL | 18 | <DL | 3.9 | 6.9 | <DL | <DL | 457 | <DL | <DL | <DL | 0 | 68 | <DL | <DL | 23.6 | 156 | 4.5 | <DL | 124 | 0.1 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 1.9 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 8.5 | <DL |
| LOP2S1 | | <DL | 37 | 18 | 2.9 | 5.5 | <DL | <DL | 514 | 0 | 0.9 | 0.1 | 5.7 | 625 | <DL | <DL | 415 | 90 | 5.1 | <DL | 99 | 18 | <DL | 0.3 | 1.8 | <DL | 0.2 | 0.3 | <DL | 2.2 | <DL | 0.5 | <DL | <DL | 0.1 | <DL | <DL | 9 | <DL |
| LOP2S2 | | <DL | 36 | 12 | 1.8 | 3.9 | <DL | <DL | 487 | 0 | 0.8 | <DL | 4.9 | 626 | <DL | <DL | 277 | 84 | 5.1 | <DL | 136 | 17 | <DL | 0.3 | 1.1 | <DL | 0.2 | 0.4 | <DL | 2 | <DL | 0.4 | <DL | <DL | 0.1 | <DL | <DL | 8.1 | <DL |
| LOP2S3 | | <DL | 18 | 0.6 | 2 | 2.7 | <DL | <DL | 270 | <DL | 0.2 | <DL | 0.1 | 42 | <DL | <DL | 24.1 | 92 | 2.9 | <DL | 122 | 1.9 | <DL | <DL | 0 | <DL | 0.1 | <DL | <DL | 1.2 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 5.5 | <DL |
| LOP2S4 | | <DL | 24 | 0.2 | 5.6 | 3.8 | <DL | <DL | 366 | <DL | 0 | <DL | 0.1 | 56 | <DL | <DL | 22 | 138 | 2.3 | <DL | 145 | 0.2 | <DL | <DL | <DL | <DL | 0.3 | <DL | <DL | 1.4 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 9.1 | <DL |
| LOP3S1 | | <DL | 35 | 5.6 | 2 | 3.1 | <DL | <DL | 314 | <DL | 0.5 | <DL | 4.3 | 648 | <DL | <DL | 602 | 63 | 4.7 | <DL | 123 | 11 | <DL | 0.2 | 2.3 | <DL | 0.2 | <DL | <DL | 1.3 | <DL | 0.4 | <DL | <DL | 0.1 | <DL | <DL | 7.1 | <DL |
| LOP3S2 | | <DL | 46 | 7 | 2.6 | 5.2 | <DL | <DL | 614 | <DL | 0.8 | <DL | 7.5 | 213 | <DL | <DL | 385 | 107 | 7.1 | <DL | 213 | 17 | <DL | 0.2 | 2.3 | <DL | 0.1 | <DL | <DL | 2.6 | <DL | 0.6 | <DL | <DL | <DL | <DL | <DL | 9.7 | <DL |
| LOP3S3 | | <DL | 15 | 0.3 | 2.2 | 4.2 | <DL | <DL | 280 | <DL | 0.2 | <DL | 0.1 | 43 | <DL | <DL | 23 | 92 | 2.7 | <DL | 142 | 1.4 | <DL | <DL | 0 | <DL | 0.1 | <DL | <DL | 1.4 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 6.3 | <DL |
| LOP3S4 | | <DL | 22 | 0.2 | 3 | 5.9 | <DL | <DL | 390 | <DL | 0.1 | <DL | 0.1 | 61 | <DL | <DL | 27.3 | 157 | 2.5 | <DL | 160 | 0.5 | <DL | <DL | <DL | <DL | 0.3 | <DL | <DL | 1.9 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 7.2 | <DL |

**Table 7.24-** Peat metal concentration (mg/kg) for the high-impacted sites along the Sudbury metal gradient.

| Peat | Sites | Ag | Al | As | B | Ba | Be | Bi | Ca | Cd | Co | Cr | Cu | Fe | Hg | In | K | Mg | Mn | Mo | Na | Ni | P | Pb | Rb | Sb | Sc | Se | Sn | Sr | Th | Ti | Tl | U | V | W | Y | Zn | Zr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CONTROL A | | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 0.1 | <DL | <DL | <DL | 0.4 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 0.1 | <DL |
| CONTROL B | | <DL | <DL | <DL | 0 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL |
| CLP1S1 | Clearwater | <DL | 51 | 0.9 | 1.3 | 2.1 | <DL | <DL | 87 | <DL | 0.2 | 0.1 | 4.6 | 157 | <DL | <DL | 198 | 13 | 0.7 | <DL | 109 | 5.4 | <DL | 0.2 | 0.2 | <DL | 0.4 | 0.3 | <DL | 0.5 | <DL | 0.9 | <DL | <DL | 0.1 | <DL | <DL | 3.8 | <DL |
| CLP1S2 | | <DL | 21 | 4.9 | 2.9 | 1.5 | <DL | <DL | 176 | <DL | 0.1 | <DL | 2.6 | 41 | <DL | <DL | 79 | 31 | 1.1 | <DL | 147 | 3.7 | <DL | 0.1 | 0 | <DL | 0.3 | <DL | <DL | 1.1 | <DL | 0.9 | <DL | <DL | 0.1 | <DL | <DL | 4.4 | <DL |
| CLP1S3 | | <DL | 14 | 1.4 | 2.6 | 2.1 | <DL | <DL | 299 | <DL | 0.1 | <DL | 0.1 | 46 | <DL | <DL | 7.1 | 58 | 1.5 | <DL | 141 | 1.1 | <DL | <DL | 0 | <DL | 0.2 | <DL | <DL | 1.4 | <DL | 0.6 | <DL | <DL | <DL | <DL | <DL | 5.4 | <DL |
| CLP1S4 | | <DL | 11 | 0.3 | 2.8 | 2.5 | <DL | <DL | 637 | <DL | 0 | <DL | 0.1 | 93 | <DL | <DL | 3.6 | 170 | 2.3 | <DL | 137 | 0.2 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 3 | <DL | 0.4 | <DL | <DL | 0.3 | <DL | <DL | 5 | <DL |
| CLP2S1 | | <DL | 19 | 2.3 | 1.7 | 2.1 | <DL | <DL | 71 | <DL | 0.1 | 0.1 | 3.3 | 83 | <DL | <DL | 304 | 8.3 | 0.3 | <DL | 162 | 5 | <DL | 0.1 | 0.5 | <DL | 0.4 | <DL | <DL | 0.4 | <DL | 0.5 | <DL | <DL | 0.1 | <DL | <DL | 4.6 | <DL |
| CLP2S2 | | <DL | 30 | 6.7 | 3.4 | 1.5 | <DL | <DL | 104 | <DL | 0.1 | <DL | 3.8 | 54 | <DL | <DL | 111 | 21 | 0.5 | <DL | 173 | 4.2 | <DL | 0.1 | 0.2 | <DL | 0.5 | <DL | <DL | 0.5 | <DL | 1.1 | <DL | <DL | 0.3 | <DL | <DL | 5.5 | <DL |
| CLP2S3 | | <DL | 17 | 1.5 | 1.5 | 2.7 | <DL | <DL | 188 | <DL | 0.1 | <DL | 0.2 | 31 | <DL | <DL | 9.1 | 43 | 1.2 | <DL | 123 | 1.4 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 0.9 | <DL | 0.7 | <DL | <DL | 0.1 | <DL | <DL | 4.1 | <DL |
| CLP2S4 | | <DL | 9.2 | 0.2 | 2.9 | 2.5 | <DL | <DL | 670 | <DL | 0 | <DL | 0.1 | 99 | <DL | <DL | <DL | 200 | 3.3 | <DL | 124 | 0.2 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 3.2 | <DL | 0.4 | <DL | <DL | 0.3 | <DL | <DL | 4.3 | <DL |
| CLP3S1 | | <DL | 28 | 1.7 | 2.7 | 2.1 | <DL | <DL | 260 | <DL | 0.2 | <DL | 3 | 69 | <DL | <DL | 356 | 55 | 4.1 | <DL | 149 | 5 | <DL | 0 | 0.4 | <DL | 0.3 | <DL | <DL | 1.6 | <DL | 0.3 | <DL | <DL | 0.1 | <DL | <DL | 5.1 | <DL |
| CLP3S2 | | <DL | 15 | 8 | 2.7 | 2.1 | <DL | <DL | 267 | <DL | 0.1 | <DL | 2.1 | 50 | <DL | <DL | 125 | 57 | 1.5 | <DL | 164 | 3.7 | <DL | 0 | 0.1 | <DL | 0.3 | <DL | <DL | 1.7 | <DL | 0.6 | <DL | <DL | <DL | <DL | <DL | 5.4 | <DL |
| CLP3S3 | | <DL | 8.9 | 8.2 | 1.8 | 4.2 | <DL | <DL | 578 | <DL | 0.2 | <DL | 0.4 | 88 | <DL | <DL | 19 | 126 | 2.9 | <DL | 144 | 2.4 | <DL | <DL | 0 | <DL | 0.1 | <DL | <DL | 3.2 | <DL | 0.5 | <DL | <DL | <DL | <DL | <DL | 4.9 | <DL |
| CLP3S4 | | <DL | 9.2 | 0.1 | 1.5 | 3.4 | <DL | <DL | 581 | <DL | 0 | <DL | 0.1 | 84 | <DL | <DL | <DL | 189 | 3 | <DL | 100 | 0.1 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 2.5 | <DL | 0.4 | <DL | <DL | 0.2 | <DL | <DL | 4.3 | <DL |
| LLP1S1 | Lake Laurentian | <DL | 27 | 8.6 | 2.1 | 4.6 | <DL | <DL | 372 | 0.1 | 0.9 | <DL | 13 | 76 | <DL | <DL | 451 | 96 | 6.1 | <DL | 130 | 22 | <DL | 0.1 | 1.1 | <DL | 0.3 | 0.2 | <DL | 1.3 | <DL | <DL | 0.1 | <DL | 0.1 | <DL | <DL | 8 | <DL |
| LLP1S2 | | <DL | 18 | 9.9 | 4.9 | 8.4 | <DL | <DL | 317 | <DL | 0.5 | <DL | 7 | 59 | <DL | <DL | 108 | 58 | 2.4 | <DL | 141 | 15 | <DL | 0.1 | 0.1 | <DL | 0.2 | 0.2 | <DL | 1.2 | <DL | 0.5 | <DL | <DL | <DL | <DL | <DL | 11 | <DL |
| LLP1S3 | | <DL | 11 | 1.1 | 1.6 | 2.9 | <DL | <DL | 489 | <DL | 0.1 | <DL | 0.1 | 75 | <DL | <DL | 14 | 115 | 1.2 | <DL | 57 | 1.1 | <DL | <DL | 0 | <DL | 0.1 | <DL | <DL | 1.5 | <DL | 0.2 | <DL | <DL | <DL | <DL | <DL | 3.9 | <DL |
| LLP1S4 | | <DL | 26 | 0.4 | 4.5 | 3.1 | <DL | <DL | 649 | <DL | 0 | <DL | 0.1 | 98 | <DL | <DL | 30 | 164 | 0.9 | <DL | 97 | 0.2 | <DL | <DL | 0 | <DL | 0.5 | <DL | <DL | 1.8 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 6.1 | <DL |
| LLP2S1 | | <DL | 35 | 1.1 | 1.5 | 4.5 | <DL | <DL | 531 | 0 | 1.3 | <DL | 5.4 | 139 | <DL | <DL | 299 | 122 | 12 | <DL | 135 | 25 | <DL | 0.2 | 0.5 | <DL | 0.2 | <DL | <DL | 1.7 | <DL | 0.3 | <DL | <DL | <DL | <DL | <DL | 8 | <DL |
| LLP2S2 | | <DL | 27 | 3.5 | 2.1 | 4.3 | <DL | <DL | 324 | <DL | 0.5 | <DL | 7.4 | 69 | <DL | <DL | 109 | 57 | 2.6 | <DL | 95 | 15 | <DL | 0.2 | 0.1 | <DL | 0.2 | <DL | <DL | 1.1 | <DL | 0.8 | <DL | <DL | 0.1 | <DL | <DL | 6.2 | <DL |
| LLP2S3 | | <DL | 7.6 | 0.4 | 1.6 | 2.7 | <DL | <DL | 725 | <DL | 0 | <DL | 0.1 | 110 | <DL | <DL | 14 | 171 | 1.2 | <DL | 59 | 0.4 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 1.9 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 3 | <DL |
| LLP2S4 | | <DL | 14 | 0.1 | 2 | 3.3 | <DL | <DL | 943 | <DL | 0 | <DL | 0.1 | 146 | <DL | <DL | 22 | 194 | 1.7 | <DL | 64 | 0.3 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 2.3 | <DL | <DL | <DL | <DL | 0.1 | <DL | <DL | 4.7 | <DL |
| LLP3S1 | | <DL | 40 | 1.7 | 1.6 | 4.9 | <DL | <DL | 547 | 0.1 | 1.3 | 0.1 | 7.8 | 161 | <DL | <DL | 629 | 143 | 5.3 | <DL | 215 | 28 | <DL | 0.1 | 2.3 | <DL | 0.3 | <DL | <DL | 1.8 | <DL | 0.5 | <DL | <DL | 0.1 | <DL | <DL | 8.6 | <DL |
| LLP3S2 | | <DL | 15 | 13 | 2.5 | 11 | <DL | <DL | 790 | 0 | 1 | <DL | 4.6 | 128 | <DL | <DL | 85 | 119 | 5.1 | <DL | 87 | 25 | <DL | <DL | 0.1 | <DL | 0.1 | <DL | <DL | 2.8 | <DL | 0.4 | <DL | <DL | <DL | <DL | <DL | 9.5 | <DL |
| LLP3S3 | | <DL | 9.8 | 1.3 | 1.3 | 2.9 | <DL | <DL | 730 | <DL | 0 | <DL | 0 | 113 | <DL | <DL | 13 | 146 | 1.2 | <DL | 47 | 0.4 | <DL | <DL | <DL | <DL | 0.1 | <DL | <DL | 2.2 | <DL | 0.2 | <DL | <DL | <DL | <DL | <DL | 2.9 | <DL |
| LLP3S4 | | <DL | 18 | 0.2 | 2 | 3.8 | <DL | <DL | 987 | <DL | 0 | <DL | 0.2 | 159 | <DL | <DL | 18 | 203 | 1.7 | <DL | 70 | 0.3 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 2.7 | <DL | 0.3 | <DL | <DL | 0.1 | <DL | <DL | 3.6 | <DL |
| WHP1S1 | Whitson | <DL | 149 | 0.6 | 2.9 | 7.5 | <DL | <DL | 706 | <DL | 1.1 | 0.4 | 7.9 | 294 | <DL | <DL | 341 | 295 | 45 | <DL | 153 | 20 | <DL | 0.4 | 1.1 | <DL | 1.8 | <DL | <DL | 2.7 | <DL | 4 | <DL | <DL | 0.2 | <DL | 0 | 6.9 | <DL |
| WHP1S2 | | <DL | 21 | 1.5 | 1.9 | 3 | <DL | <DL | 186 | <DL | 0.1 | <DL | 2.6 | 33 | <DL | <DL | 343 | 74 | 1.9 | <DL | 88 | 5.6 | <DL | 0.2 | 0.7 | <DL | 0.3 | <DL | <DL | 0.7 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 4.8 | <DL |
| WHP1S3 | | <DL | 11 | 2.4 | 1.6 | 3.6 | <DL | <DL | 453 | <DL | 0.1 | <DL | 0.2 | 68 | <DL | <DL | 23 | 154 | 6.5 | <DL | 69 | 3.7 | <DL | <DL | 0 | <DL | 0.3 | <DL | <DL | 1.9 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 4 | <DL |
| WHP1S4 | | <DL | 12 | 0.1 | 0.5 | 1.2 | <DL | <DL | 194 | <DL | 0 | <DL | 0.1 | 32 | <DL | <DL | 2.8 | 53 | 0.7 | <DL | 21 | 0.1 | <DL | <DL | <DL | <DL | 0.2 | <DL | <DL | 0.6 | <DL | 0.5 | <DL | <DL | 0.3 | <DL | 0 | 0.9 | <DL |
| WHP2S1 | | <DL | 42 | 0.4 | 2.8 | 6 | <DL | <DL | 621 | <DL | 1.6 | 0.2 | 6.3 | 159 | <DL | <DL | 667 | 260 | 52 | <DL | 172 | 20 | <DL | 0.1 | 2.1 | <DL | 1 | <DL | <DL | 2.4 | <DL | 0.6 | <DL | <DL | <DL | <DL | <DL | 7.3 | <DL |
| WHP2S2 | | <DL | 51 | 2.6 | 2.5 | 3.8 | <DL | <DL | 223 | <DL | 0.1 | <DL | 4.7 | 52 | <DL | <DL | 213 | 87 | 2.9 | <DL | 113 | 6.7 | <DL | 0.1 | 0.5 | <DL | 0.8 | <DL | <DL | 0.8 | <DL | 1.1 | <DL | <DL | 0.2 | <DL | <DL | 5.4 | <DL |
| WHP2S3 | | <DL | 16 | 1.3 | 1.4 | 5.7 | <DL | <DL | 479 | <DL | 0.2 | <DL | 0.2 | 74 | <DL | <DL | 18 | 167 | 7.4 | <DL | 61 | 7.6 | <DL | <DL | 0 | <DL | 0.3 | <DL | <DL | 2.3 | <DL | 0.6 | <DL | <DL | <DL | <DL | <DL | 4.7 | <DL |
| WHP2S4 | | <DL | 6.3 | 0.3 | 1.7 | 1.6 | <DL | <DL | 412 | <DL | 0 | <DL | 0.1 | 61 | <DL | <DL | 4.2 | 112 | 2.9 | <DL | 54 | 0.4 | <DL | <DL | <DL | <DL | 0.3 | <DL | <DL | 1.4 | <DL | 0.2 | <DL | <DL | 0.2 | <DL | <DL | 1.9 | <DL |
| WHP3S1 | | <DL | 87 | 0.5 | 3.4 | 9 | <DL | <DL | 642 | <DL | 0.8 | <DL | 4.8 | 161 | <DL | <DL | 229 | 267 | 24 | <DL | 140 | 16 | <DL | 0.1 | 0.6 | <DL | 1.1 | <DL | <DL | 2.7 | <DL | 1.8 | <DL | <DL | <DL | <DL | <DL | 9.6 | <DL |
| WHP3S2 | | <DL | 25 | 3.6 | 1.5 | 5.6 | <DL | <DL | 522 | <DL | 0.3 | <DL | 2.3 | 81 | <DL | <DL | 180 | 199 | 8.3 | <DL | 84 | 9 | <DL | <DL | 0.8 | <DL | 0.2 | <DL | <DL | 2.3 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 6.3 | <DL |
| WHP3S3 | | <DL | 9.5 | 2.7 | 1.1 | 3.4 | <DL | <DL | 431 | <DL | 0.2 | <DL | 0.2 | 62 | <DL | <DL | 35 | 132 | 5.9 | <DL | 63 | 6.5 | <DL | <DL | 0 | <DL | 0.2 | <DL | <DL | 2 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | 4.4 | <DL |
| WHP3S4 | | <DL | 4.4 | 0.1 | 0.7 | 2 | <DL | <DL | 367 | <DL | 0 | <DL | 0.1 | 56 | <DL | <DL | 4.1 | 99 | 1.8 | <DL | 43 | 0.1 | <DL | <DL | <DL | <DL | 0.1 | <DL | <DL | 1.3 | <DL | 0.1 | <DL | <DL | 0.2 | <DL | 0 | 1.9 | <DL |

**Table 7.25-** Peat metal concentration groupings and cut off based on mean [Cu] in the sites along the Sudbury metal gradient.

| Category | Mean [Cu] mg/kg | Mean [Ni] mg/kg |
|---|---|---|
| Low-impacted | 0.1898-0.2753 | 0.9921-1.880 |
| Intermediate-low impacted | 0.2319-0.7443 | 2.577-6.711 |
| Intermediate-high impacted | 0.9684-1.609 | 1.567-2.548 |
| High-impacted | 2.372-2.609 | 6.816-8.005 |

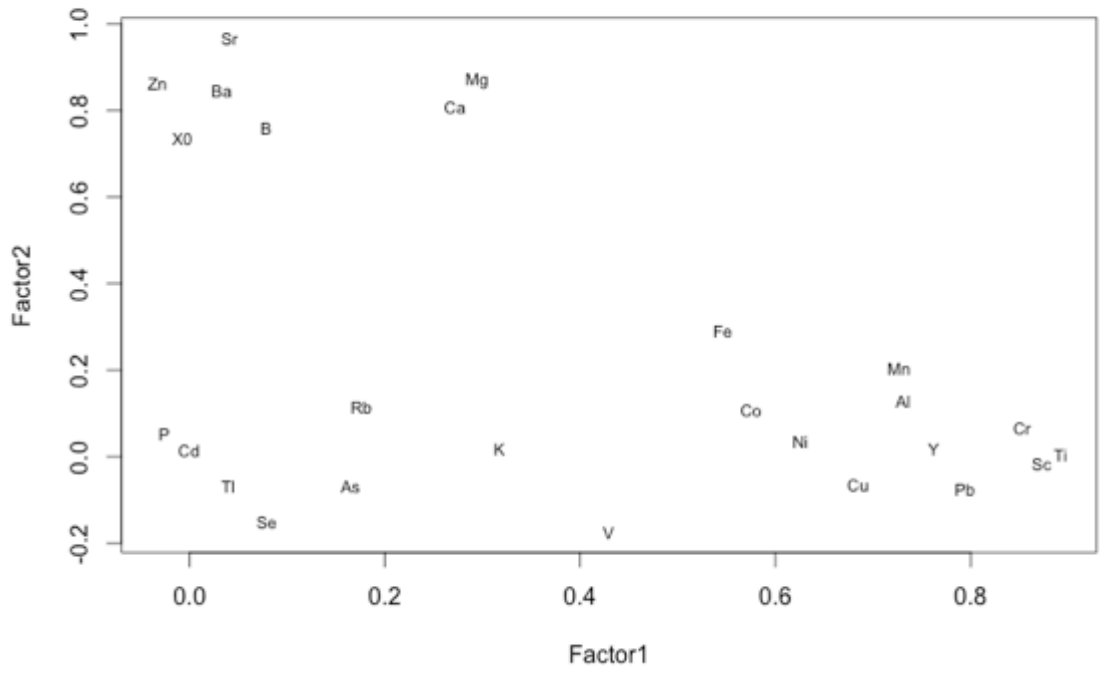



**Figure 7.39-** Factorial analysis on chemical data to determine the range of distribution of the data and the representative chemicals to be used for further analysis.

## 7.5 Chemistry Analysis

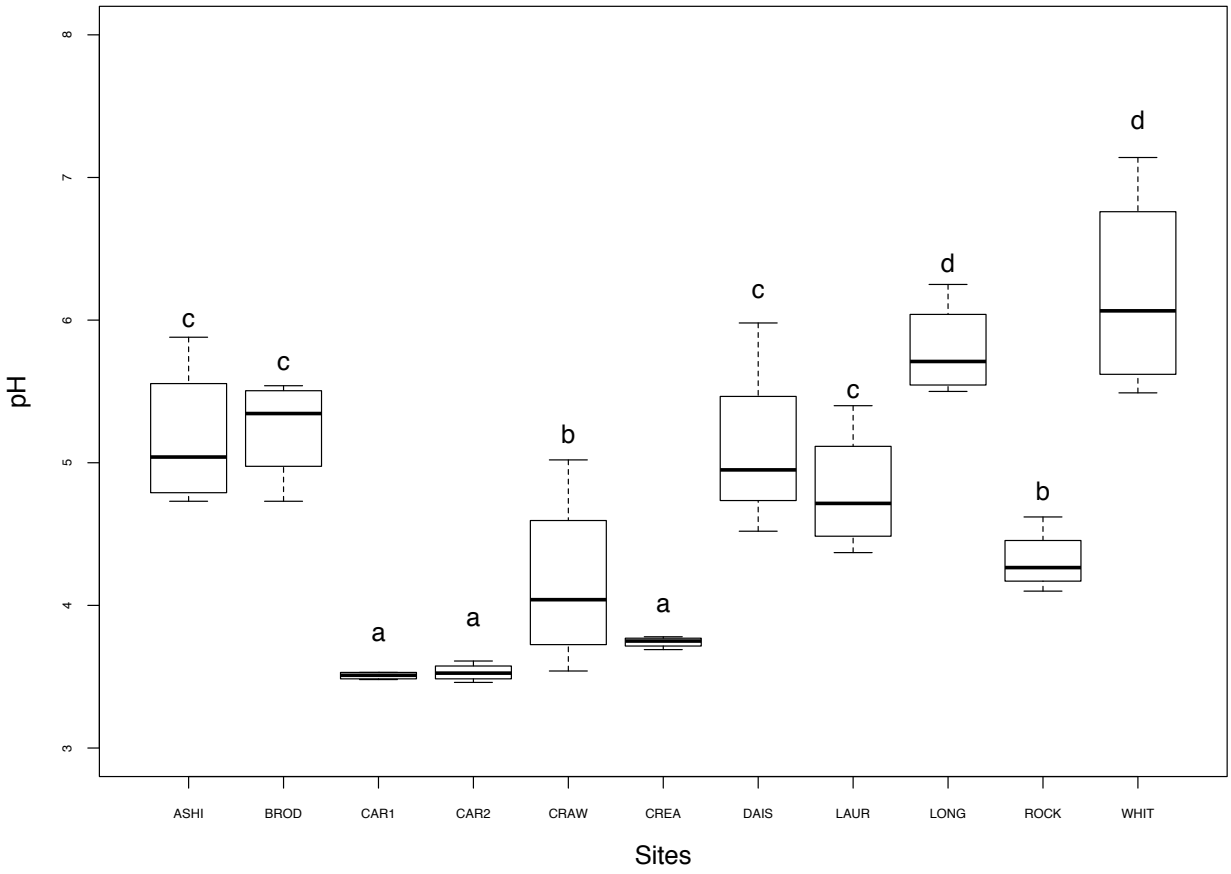



**Figure 7.40**- Box plots of pH values within peat samples across the study sites with the results of Post-hoc tests, where the letters represent significant differences. Sites with shared letters show no significant difference. ANOVA results for pH versus site distance was observed with *p*-value = 0.000308*** and F = 40.87 (Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1). Error bars represent standard deviation of the mean.

## 7.6 Peatland Ecology

### Table 7.26- Plant characteristics across study sites including vascular and non-vascular species

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Broder** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Chamaedaphne calyculata* | Shrub | | 50 | | *Chamaedaphne calyculata* | Shrub | | 50 | | *Chamaedaphne calyculata* | Shrub | | 20 | |
| | *Ledum groenlandicum* | Shrub | | 5 | | *Juncus sp.* | Rushes | | 5 | | *Ledum groenlandicum* | Shrub | | 5 | |
| | *Dulichium arundinaceum* | Sedge | | 10 | | *Carex sp.* | Sedge | | 5 | | *Dulichium arundinaceum* | Sedge | | 15 | |
| | *Eriophorum vaginatum* | Sedge | | 1 | | *Dulichium arundinaceum* | Sedge | | 5 | | Unknown sedge | Sedge | | 15 | |
| | *Carex sp.* | Sedge | | 5 | | | | | | | | | | | |
| | Sphagnum | Bryophyte | | 2 | 1 | Sphagnum | Bryophyte | | 30 | 2 | Sphagnum | Bryophyte | | 0 | 0 |
| | Other moss | Bryophyte | | | 1 | Other moss | Bryophyte | | | 1 | Other moss | Bryophyte | | | 1 |
| | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 |
| **Clearwater** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Chamaedaphne calyculata* | Shrub | | 80 | | *Chamaedaphne calyculata* | Shrub | | 80 | | *Chamaedaphne calyculata* | Shrub | | 70 | |
| | *Eriophorum spicum* | Sedge | | 10 | | Unknown sedge | Sedge | | 10 | | Unknown sedge | Sedge | | 15 | |
| | Sphagnum | Bryophyte | sparse | | 0 | Sphagnum | Bryophyte | | | 0 | Sphagnum | Bryophyte | | | 0 |
| | Other moss | Bryophyte | 5 – 10 | | 1 | Other moss | Bryophyte | | | 0 | Other moss | Bryophyte | | | 0 |
| | Lichen | Ascolichens/ Basidiolichens | | 0 | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 |
| **Cartier1** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Ledum groenlandicum* | Shrub | | 30 | | *Ledum groenlandicum* | Shrub | | 40 | | *Ledum groenlandicum* | Shrub | | 40 | |
| | *Chamaedaphne calyculata* | Shrub | | 20 | | *Chamaedaphne calyculata* | Shrub | | 5 | | *Chamaedaphne calyculata* | Shrub | | 10 | |
| | *Kalmia polifolia* | Shrub | | 10 | | *Kalmia polifolia* | Shrub | | 5 | | *Kalmia polifolia* | Shrub | | 5 | |
| | *Vaccinium sp.* | Shrub | | 10 | | *Eriophorum spicum* | Sedge | | 5 | | *Eriophorum sp.* | Sedge | | 5 | |
| | *Eriophorum sp.* | Sedge | | 5 | | | | | | | Unknown lily plant | Herb | | 20 | |
| | Various fungi | NA | | 5 | | | | | | | | | | | |
| | Unknown lily plant | Herb | | 5 | | | | | | | | | | | |
| | Sphagnum | Bryophyte | | 80 | 3 | Sphagnum | Bryophyte | | 90 | 3 | Sphagnum | Bryophyte | | 80 | 3 |
| | Other moss | Bryophyte | | | 0 | Other moss | Bryophyte | | | 0 | Other moss | Bryophyte | | | 0 |
| | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 |
| **Cartier2** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Sarracenia purpurea* | Herb | | 5 | | *Sarracenia purpurea* | Herb | | 5 | | *Kalmia polifolia* | Shrub | | 5 | |
| | *Chamaedaphne calyculata* | Shrub | | 10 | | *Chamaedaphne calyculata* | Shrub | | 5 | | *Chamaedaphne calyculata* | Shrub | | 5 | |
| | *Kalmia polifolia* | Shrub | | 5 | | *Vaccinium sp.* | Shrub | | 5 | | *Vaccinium sp.* | Shrub | | 5 | |
| | *Eriophorum spicum* | Sedge | | 25 | | *Eriophorum spicum* | Sedge | | 25 | | *Eriophorum spicum* | Sedge | | 40 | |
| | *Vaccinium sp.* | Shrub | | 5 | | | | | | | | | | | |
| | Sphagnum | Bryophyte | | 40 | 3 | Sphagnum (angustifolium, rubellum, cap | Bryophyte | | 90 | 3 | Sphagnum | Bryophyte | | 80 | 3 |
| | Other moss | Bryophyte | | | 0 | Other moss | Bryophyte | | | 0 | Other moss | Bryophyte | | | 0 |
| | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 |
| | Black spruce | Tree | | | | Black spruce | Tree | | | | Black spruce | Tree | | | |
| | Stunted tamarck | Tree | | | | Stunted tamarack | Tree | | | | Alder | Tree | | | |
| **Rockcut** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Myrica gale* | Shrub | | 30 | | *Myrica gale* | Shrub | | 10 | | *Myrica gale* | Shrub | | 50 | |
| | *Chamaedaphne calyculata* | Shrub | | 10 | | Unknown shrub | Shrub | | 40 | | *Chamaedaphne calyculata* | Shrub | | 5 | |
| | Unknown grasses | Grass | | 30 | | Unknown grasses | Grass | | 30 | | Unknown grasses | Grass | | 15 | |
| | *Eriophorum spicum* | Sedge | | 5 | | *Eriophorum spicum* | Sedge | | 5 | | | | | | |
| | Sphagnum (angustifolium) | Bryophyte | | 70 | 1 | Sphagnum (angustifolium and Polytricur | Bryophyte | | 60 | 2 | Sphagnum | Bryophyte | | 70 | 2 |
| | Other moss | Bryophyte | | | 0 | Other moss | Bryophyte | | | 0 | Other moss | Bryophyte | | | 0 |
| | Lichen | Ascolichens/ Basidiolichens | | | 1 | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 |
| | Black spruce | Tree | | | | Black spruce | Tree | | | | Black spruce | Tree | | | |
| | Alder | Tree | | | | Alder | Tree | | | | Jack pine | Tree | | | |
| | | | | | | White birch | Tree | | | | | | | | |
| **Whitson** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Carex sp.* | Sedge | | 40 | | *Carex sp.* | Sedge | | 40 | | *Carex sp.* | Sedge | | 40 | |
| | *Hypericum sp.* | Shrub | | 5 | | *Hypericum sp.* | Shrub | | 5 | | *Chamaedaphne calyculata* | Shrub | | 10 | |
| | | | | | | | | | | | *Hypericum sp.* | Shrub | | 5 | |
| | Sphagnum | Bryophyte | | 50 | 1 | Sphagnum | Bryophyte | | 40 | 1 | Sphagnum | Bryophyte | | 35 | 1 |
| | Other moss | Bryophyte | | | 0 | Other moss | Bryophyte | | | 0 | Other moss | Bryophyte | | | 0 |
| | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 |
| **Ashigami** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Typha sp.* | Grass | | 5 | | *Typha sp.* | Grass | | 5 | | *Typha sp.* | Grass | | 10 | |
| | *Chamaedaphne calyculata* | Shrub | | 30 | | *Chamaedaphne calyculata* | Shrub | | 5 | | *Chamaedaphne calyculata* | Shrub | | 30 | |
| | Unknown sedge | Sedge | | 10 | | Unknown sedge | Sedge | | 20 | | Unknown sedge | Sedge | | 25 | |
| | *Drosera rotundifolia* | Herb | | 40 | | *Drosera rotundifolia* | Herb | | 5 | | *Drosera rotundifolia* | Herb | | 30 | |
| | *Juncus sp.* | Rushes | | 5 | | *Eriophorum sp.* | Sedge | | 5 | | *Carex sp.* | Sedge | | 5 | |
| | | | | | | *Carex sp.* | Sedge | | 5 | | | | | | |
| | | | | | | *Kalmia angustifolia* | Shrub | | 5 | | | | | | |
| | Sphagnum | Bryophyte | | 50 | 2 | Sphagnum | Bryophyte | | 40 | 2 | Sphagnum | Bryophyte | | 25 | 2 |
| | Other moss | Bryophyte | | | 1 | Other moss | Bryophyte | | | 1 | Other moss | Bryophyte | | | 1 |
| | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 2 | Lichen | Ascolichens/ Basidiolichens | | | 0 |
| | Alder | Tree | | | | Alder | Tree | | | | Alder | Tree | | | |
| | willow | Tree | | | | Black spruce | Tree | | | | Black spruce | Tree | | | |
| | birch | Tree | | | | Birch | Tree | | | | Birch | Tree | | | |
| **Lake Laurentian** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Eriophorum vaginatum* | Sedge | | 10 | | *Eriophorum vaginatum* | Sedge | | 10 | | *Eriophorum vaginatum* | Sedge | | 10 | |
| | *Chamaedaphne calyculata* | Shrub | | 70 | | *Chamaedaphne calyculata* | Shrub | | 50 | | *Chamaedaphne calyculata* | Shrub | | 30 | |
| | Unknown sedge | Sedge | | 30 | | Unknown sedge | Sedge | | 30 | | Unknown sedge | Sedge | | 40 | |
| | *Maianthemum trifolium* | Herb | | 10 | | Unknown grasses | Grass | | 5 | | | | | | |
| | | | | | | *Carex sp.* | Sedge | | 5 | | Un-vegetated peat | | | 15 | |
| | bare/unvegetated peat | | | 10 | | Un-vegetated surface | | | 15 | | | | | | |
| | Sphagnum | Bryophyte | | | 0 | Sphagnum | Bryophyte | | | 0 | Sphagnum | Bryophyte | | | 0 |
| | Other moss | Bryophyte | | 10 | 1 | Other moss | Bryophyte | | 30 | 1 | Other moss | Bryophyte | | 40 | 1 |
| | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 |
| **Crowley** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Eriophorum vaginatum* | Sedge | | 35 | | *Eriophorum vaginatum* | Sedge | | 5 | | *Carex sp.* | Sedge | | 10 | |
| | *Chamaedaphne calyculata* | Shrub | | 50 | | *Chamaedaphne calyculata* | Shrub | | 60 | | *Chamaedaphne calyculata* | Shrub | | 80 | |
| | *Kalmia polifolia* | Shrub | | 15 | | *Kalmia polifolia* | Shrub | | 10 | | *Kalmia polifolia* | Shrub | | 10 | |
| | *Salix pedicellaris* | Shrub | | 25 | | *Salix pedicellaris* | Shrub | | 20 | | *Salix pedicellaris* | Shrub | | 10 | |
| | | | | | | *Ledum groenlandicum* | Shrub | | 10 | | *Ledum groenlandicum* | Shrub | | 10 | |
| | Sphagnum | Bryophyte | | 0 | 0 | Sphagnum | Bryophyte | | 0 | 0 | Sphagnum | Bryophyte | | 0 | 1 |
| | Other moss | Bryophyte | | | 1 | Other moss | Bryophyte | | | 2 | Other moss | Bryophyte | | | 2 |
| | Lichen | Ascolichens/ Basidiolichens | | | 2 | Lichen | Ascolichens/ Basidiolichens | | | 2 | Lichen | Ascolichens/ Basidiolichens | | | 2 |
| **Long Lake** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Carex sp.* | Sedge | | 30 | | *Carex sp.* | Sedge | | 80 | | *Carex sp.* | Sedge | | 30 | |
| | *Chamaedaphne calyculata* | Shrub | | 30 | | | | | | | *Chamaedaphne calyculata* | Shrub | | 40 | |
| | *Alnus incana* | Shrub | | 10 | | *Alnus incana* | Shrub | | 30 | | *Alnus incana* | Shrub | | 20 | |
| | Sphagnum | Bryophyte | | 10 | 1 | Sphagnum | Bryophyte | | 20 | 1 | Sphagnum | Bryophyte | | 15 | 1 |
| | Other moss | Bryophyte | | | 0 | Other moss | Bryophyte | | | 3 | Other moss | Bryophyte | | | 1 |
| | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 |
| | | | | | | *Polytricum spp.* | Bryophyte | | 5 | | | | | | |
| **Daisy Lake I** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** | **Plants** | **Functional/morphological group** | **% cover** | | **Richness** |
| | *Eriophorum spicum* | Sedge | | 80 | | *Eriophorum spicum* | Sedge | | 80 | | *Eriophorum spicum* | Sedge | | 80 | |
| | *Unknown grasses* | Grass | | 15 | | *Unknown grasses* | Grass | | 15 | | *Unknown grasses* | Grass | | 15 | |
| | *Glyceria sp.* | Grass | | 5 | | | | | | | *Glyceria* | Grass | | 5 | |
| | Sphagnum | Bryophyte | | 0 | 1 | Sphagnum | Bryophyte | | 0 | 0 | Sphagnum | Bryophyte | | 0 | 0 |
| | Other moss | Bryophyte | | | 1 | Other moss | Bryophyte | | | 1 | Other moss | Bryophyte | | | 1 |
| | Lichen | Ascolichens/Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 | Lichen | Ascolichens/ Basidiolichens | | | 0 |
| | *Polytricum spp.* | Bryophyte | | 10 | | *Polytricum spp.* | Bryophyte | | 25 | | *Polytricum spp.* | Bryophyte | | 20 | |
| | White birch | Tree | | | | White birch | Tree | | | | White birch | Tree | | | |
| | Red maple | Tree | | | | Red maple | Tree | | | | Red maple | Tree | | | |

**Table 7.27-** The interaction between plant communities and the key microbial community with the indicator microbial species, across the studied peatlands using Pearson correlation, where Corr. represents the correlation coefficient and the *p*-value represents the statistical significance of the interaction.

| OTU# | *Camaedaphne calyculata* | | *Ledum groenlandicum* | | *Eriophorum* sp. | | *Carex* sp. | | *Sarracenia purpurea* | | *Myrica gale* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Corr. | p- value | Corr. | p-value | Corr. | p-value | Corr. | p-value | Corr. | p-value | Corr. | p-value |
| OTU1 | 0.247 | 0.464 | 0.302 | 0.366 | 0.062 | 0.857 | -0.343 | 0.301 | 0.358 | 0.279 | 0.051 | 0.882 |
| OTU10 | 0.370 | 0.263 | -0.188 | 0.579 | 0.023 | 0.947 | -0.254 | 0.452 | 0.528 | 0.095 | -0.147 | 0.667 |
| OTU11 | -0.102 | 0.766 | 0.631 | 0.037* | -0.288 | 0.391 | -0.256 | 0.447 | -0.192 | 0.573 | -0.192 | 0.573 |
| OTU12 | -0.258 | 0.445 | -0.128 | 0.707 | 0.232 | 0.493 | -0.173 | 0.611 | 1.00 | 0.000*** | -0.100 | 0.770 |
| OTU14 | -0.258 | 0.445 | -0.128 | 0.707 | 0.232 | 0.493 | -0.173 | 0.611 | 1.00 | 0.000*** | -0.100 | 0.770 |
| OTU15 | 0.618 | 0.043* | -0.128 | 0.707 | -0.140 | 0.681 | -0.173 | 0.611 | -0.100 | 0.770 | -0.100 | 0.770 |
| OTU16 | -0.279 | 0.406 | -0.128 | 0.707 | -0.140 | 0.681 | -0.173 | 0.611 | -0.100 | 0.770 | 1.00 | 0.000*** |
| OTU2 | 0.019 | 0.957 | -0.010 | 0.977 | 0.111 | 0.745 | -0.289 | 0.389 | 0.502 | 0.116 | 0.180 | 0.597 |
| OTU3 | -0.024 | 0.945 | -0.099 | 0.773 | 0.660 | 0.027* | -0.285 | 0.395 | 0.509 | 0.109 | -0.192 | 0.572 |
| OTU4 | -0.103 | 0.764 | 0.446 | 0.169 | -0.026 | 0.940 | -0.392 | 0.233 | 0.590 | 0.056. | 0.1586 | 0.641 |
| OTU5 | 0.384 | 0.244 | -0.302 | 0.366 | -0.054 | 0.875 | 0.020 | 0.954 | 0.464 | 0.150 | -0.236 | 0.486 |
| OTU6 | -0.176 | 0.605 | 0.487 | 0.129 | 0.072 | 0.834 | -0.313 | 0.349 | 0.625 | 0.040* | -0.191 | 0.575 |
| OTU7 | -0.078 | 0.820 | 0.770 | 0.006** | -0.227 | 0.503 | -0.306 | 0.360 | -0.177 | 0.603 | 0.364 | 0.271 |
| OTU8 | 0.097 | 0.776 | 0.346 | 0.296 | 0.022 | 0.950 | -0.331 | 0.321 | 0.627 | 0.039* | -0.191 | 0.573 |
| OTU9 | -0.511 | 0.108 | 0.333 | 0.317 | -0.120 | 0.725 | 0.091 | 0.790 | 0.472 | 0.142 | -0.238 | 0.480 |
| OTU18 | -0.220 | 0.517 | -0.191 | 0.574 | 0.016 | 0.963 | 0.454 | 0.161 | 0.622 | 0.041* | -0.149 | 0.662 |
| OTU34 | 0.177 | 0.602 | -0.191 | 0.573 | -0.172 | 0.612 | 0.433 | 0.183 | -0.149 | 0.662 | -0.149 | 0.662 |
| OTU56 | -0.050 | 0.884 | -0.128 | 0.707 | -0.186 | 0.584 | 0.731 | 0.011* | -0.100 | 0.770 | -0.100 | 0.770 |
| OTU76 | 0.221 | 0.515 | -0.186 | 0.583 | -0.148 | 0.664 | 0.298 | 0.383 | -0.145 | 0.670 | -0.145 | 0.6670 |
| OTU19 | -0.258 | 0.445 | -0.128 | 0.707 | 0.232 | 0.493 | -0.173 | 0.611 | 1.00 | 0.000*** | -0.100 | 0.770 |
| OTU20 | -0.195 | 0.566 | 0.981 | 0.000*** | -0.116 | 0.733 | -0.173 | 0.611 | -0.100 | 0.770 | -0.100 | 0.770 |
| OTU35 | -0.258 | 0.445 | -0.128 | 0.707 | 0.232 | 0.493 | -0.173 | 0.611 | 1.00 | 0.000*** | -0.100 | 0.770 |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Corr. represents the correlation coefficient of the sample estimates. A total of 12 plant species (p-value < 0.05) were represented of the 18 total plant species analyzed at the sites.

**Table 7.12 continued-**The interactions between plant communities and the key microbial community with the indicator microbial species, across the studied peatlands using Pearson correlation, where Corr. represents the correlation coefficient and the *p*-value represents the statistical significance of the interaction.

| OTU# | *Juncus* sp. | | Maianthemum trifolium | | *Vaccinium* sp. | | *Salix pedicellaris* | | *Alnus incana* | | *Sphagnum* sp. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Corr. | p-value | Corr. | p-value | Corr. | p-value | Corr. | p-value | Corr. | p-value | Corr. | p-value |
| OTU1 | -0.467 | 0.148 | -0.313 | 0.348 | 0.501 | 0.116 | 0.020 | 0.953 | -0.095 | 0.780 | 0.242 | 0.473 |
| OTU10 | -0.219 | 0.518 | -0.147 | 0.667 | 0.378 | 0.252 | -0.147 | 0.667 | -0.147 | 0.670 | -0.005 | 0.988 |
| OTU11 | 0.677 | 0.022* | -0.192 | 0.573 | 0.193 | 0.570 | -0.192 | -.573 | -0.192 | 0.573 | 0.363 | 0.272 |
| OTU12 | -0.149 | 0.662 | -0.100 | 0.770 | 0.818 | 0.002** | -0.100 | 0.770 | -0.100 | 0.770 | 0.420 | 0.199 |
| OTU14 | -0.149 | 0.662 | -0.100 | 0.770 | 0.818 | 0.002** | -0.100 | 0.770 | -0.100 | 0.770 | 0.420 | 0.199 |
| OTU15 | -0.149 | 0.662 | -0.100 | 0.770 | -0.145 | 0.670 | -0.100 | 0.770 | -0.100 | 0.770 | -0.308 | 0.357 |
| OTU16 | -0.149 | 0.662 | -0.100 | 0.770 | -0.145 | 0.670 | -0.100 | 0.770 | -0.100 | 0.770 | 0385 | 0.242 |
| OTU2 | -0.407 | 0.214 | -0.273 | 0.417 | 0.477 | 0.138 | -0.273 | 0.417 | -0.273 | 0.417 | 0.326 | 0.328 |
| OTU3 | -0.286 | 0.394 | -0.192 | 0.572 | 0.335 | 0.314 | 0.611 | 0.046* | -0.192 | 0.572 | -0.127 | 0.710 |
| OTU4 | -0.338 | 0.309 | -0.227 | 0.502 | 0.808 | 0.003** | -0.227 | 0.502 | -0.227 | 0.502 | 0.660 | 0.027* |
| OTU5 | -0.351 | 0.290 | 0.474 | 0.141 | 0.270 | 0.421 | -0.236 | 0.486 | 0.259 | 0.443 | -0.192 | 0.572 |
| OTU6 | -0.284 | 0.397 | 0.365 | 0.270 | 0.856 | 0.001** | -0.191 | 0.575 | -0.191 | 0.575 | 0.524 | 0.098 |
| OTU7 | -0.264 | 0.433 | -0.177 | 0.603 | 0.314 | 0.347 | -0.177 | 0.603 | -0.177 | 0.603 | 0.575 | 0.065. |
| OTU8 | -0.285 | 0.395 | -0.191 | 0.573 | 0.778 | 0.005** | -0.191 | 0.573 | -0.191 | 0.573 | 0.414 | 0.205 |
| OTU9 | 0.102 | 0.766 | -0.238 | 0.480 | 0.642 | 0.033* | -0.238 | 0.480 | -0.238 | 0.480 | 0.719 | 0.013* |
| OTU18 | -0.222 | 0.512 | -0.149 | 0.662 | 0.458 | 0.156 | -0.149 | 0.662 | 0.717 | 0.013* | 0.174 | 0.608 |
| OTU34 | -0.222 | 0.511 | 0.680 | 0.021* | -0.217 | 0.522 | -0.149 | 0.662 | 0.662 | 0.027* | -0.344 | 0.301 |
| OTU56 | -0.149 | 0.662 | -0.100 | 0.770 | -0.145 | 0.670 | -0.100 | 0.770 | 1.00 | 0.000*** | -0.152 | 0.656 |
| OTU76 | -0.217 | 0.522 | 0.819 | 0.002** | -0.211 | 0.533 | -0.145 | 0.670 | 0.488 | 0.127 | -0.357 | 0.281 |
| OTU19 | -0.149 | 0.662 | -0.100 | 0.770 | 0.818 | 0.002** | -0.100 | 0.770 | -0.100 | 0.770 | 0.420 | 0.199 |
| OTU20 | -0.149 | 0.662 | -0.100 | 0.770 | 0.490 | 0.126 | -0.100 | 0.770 | -0.100 | 0.770 | 0.558 | 0.075. |
| OTU35 | -0.149 | 0.662 | -0.100 | 0.770 | 0.818 | 0.002** | -0.100 | 0.770 | -0.100 | 0.770 | 0.420 | 0.199 |

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Corr. represents the correlation coefficient of the sample estimates. A total of 12 plant species (p-value < 0.05) were represented of the 18 total plant species analyzed at the sites.

## 7.7 Microbial Analysis

**Summary showing sample statistics, including microbial observations and counts**

Number of samples: 96
Number of observations: 13244
Total count: 14208425
Table density (fraction of non-zero values): 0.229

Counts/sample summary:
Min: 103325.0
Max: 184689.0
Median: 149108.500
Mean: 148004.427
Std. dev.: 16347.324

**Counts/sample detail:**

CAR100430: 103325.0
CAR100630: 106199.0
CAR100530: 106885.0
BROD00960: 109420.0
CAR200630: 114166.0
CREA00960: 116561.0
CRAW00110: 121131.0
CAR200530: 123052.0
CAR100210: 123466.0
BROD00630: 123904.0
CREA00860: 124347.0
CRAW00530: 126702.0
CREA00630: 131308.0
CAR200860: 131309.0
WHIT00530: 133081.0
CAR100310: 134006.0
ROCK00860: 134511.0
LAUR00310: 134820.0
ROCK00210: 136262.0
CRAW00860: 136317.0
CAR100760: 137084.0
CRAW00310: 138143.0
CAR100960: 138165.0
LAUR00110: 138191.0
LAUR00760: 138655.0
CREA00310: 138907.0
CAR200430: 139948.0
CRAW00760: 140296.0
ASHI00430: 140571.0
ASHI00210: 142242.0
DAIS00110: 142962.0
CRAW00430: 143330.0
BROD00860: 143915.0
BROD00530: 145010.0
CREA00210: 145644.0
CREA00760: 146029.0
CREA00110: 146366.0
LAUR00210: 146515.0
ASHI00760: 146832.0

**Counts/sample detail:**

WHIT00960: 146953.0
LONG00210: 146978.0
ASHI00530: 147437.0
LAUR00630: 147826.0
BROD00110: 147854.0
CRAW00960: 148192.0
CRAW00210: 148908.0
BROD00210: 148918.0
ROCK00760: 149082.0
CAR200960: 149135.0
CREA00430: 150556.0
LONG00310: 150934.0
WHIT00860: 151206.0
CAR200210: 151681.0
BROD00430: 151810.0
WHIT00760: 151877.0
ASHI00860: 152350.0
LONG00430: 152447.0
LAUR00860: 152654.0
CAR200310: 152724.0
LONG00860: 153341.0
BROD00760: 153726.0
CAR100110: 153929.0
CRAW00630: 154573.0
WHIT00430: 154652.0
ASHI00110: 155875.0
CAR200760: 156329.0
ROCK00110: 156634.0
WHIT00210: 157968.0
CREA00530: 158116.0
LONG00110: 159563.0
LONG00960: 159688.0
DAIS00310: 159691.0
WHIT00110: 160156.0
DAIS00630: 160164.0
ROCK00430: 160323.0
ROCK00310: 161341.0
LONG00530: 161947.0
LAUR00960: 162316.0

**Counts/sample detail:**

LONG00760: 163374.0
ROCK00630: 163542.0
DAIS00430: 163637.0
DAIS00210: 164227.0
ROCK00530: 164284.0
ASHI00960: 165008.0
WHIT00310: 165039.0
LONG00630: 165319.0
DAIS00530: 167035.0
ASHI00310: 167982.0
CAR100860: 168854.0
ASHI00630: 169528.0
BROD00310: 169753.0
WHIT00630: 171569.0
ROCK00960: 172196.0
LAUR00430: 173366.0
CAR200110: 179592.0
LAUR00530: 184689.0

**Table 7.28**- Taxonomic assignment for microbial species using GreenGenes IDs (Confidence >0.5) for the >1% data set

| OTU | Kingdom | Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|---|---|
| OTU1 | Bacteria | Acidobacteria | TM1 | Unknown | Unknown | Unknown |
| OTU10 | Bacteria | Proteobacteria | Alphaproteobacteria | Rhizobiales | Methylocystaceae | Unknown |
| OTU11 | Bacteria | Nitrospirae | Nitrospira | Nitrospirales | Thermodesulfovibrionaceae | Unknown |
| OTU12 | Bacteria | Proteobacteria | Betaproteobacteria | SBla14 | Unknown | Unknown |
| OTU14 | Bacteria | NC10 | 12-24 | JH-WHS47 | Unknown | Unknown |
| OTU15 | Bacteria | Proteobacteria | Deltaproteobacteria | Syntrophobacterales | Syntrophobacteraceae | Unknown |
| OTU16 | Bacteria | Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | Bradyrhizobium |
| OTU17 | Bacteria | Proteobacteria | Unknown | Unknown | Unknown | Unknown |
| OTU18 | Bacteria | Acidobacteria | Acidobacteriia | Acidobacteriales | Acidobacteriaceae | Unknown |
| OTU19 | Bacteria | Verrucomicrobia | Pedosphaerae | Pedosphaerales | Ellin515 | Unknown |
| OTU2 | Bacteria | Proteobacteria | Deltaproteobacteria | Syntrophobacterales | Syntrophaceae | Desulfobacca |
| OTU20 | Bacteria | Planctomycetes | Phycisphaerae | MSBL9 | Unknown | Unknown |
| OTU21 | Bacteria | Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | Unknown |
| OTU22 | Bacteria | Acidobacteria | Solibacteres | Solibacterales | Solibacteraceae | Candidatus Solibacter |
| OTU23 | Bacteria | Acidobacteria | Solibacteres | Solibacterales | AKIW659 | Unknown |
| OTU25 | Bacteria | Chloroflexi | Dehalococcoidetes | Dehalococcoidales | Unknown | Unknown |
| OTU27 | Bacteria | Actinobacteria | Thermoleophilia | Gaiellales | Gaiellaceae | Unknown |
| OTU28 | Bacteria | Actinobacteria | Thermoleophilia | Solirubrobacterales | Unknown | Unknown |
| OTU3 | Archaea | Euryarchaeota | Thermoplasmata | E2 | Methanomassiliicoccaceae | Unknown |
| OTU30 | Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Unknown | Unknown |
| OTU34 | Bacteria | Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | Candidatus Koribacter |
| OTU35 | Bacteria | Proteobacteria | Deltaproteobacteria | Syntrophobacterales | Syntrophaceae | Syntrophus |
| OTU36 | Bacteria | Actinobacteria | Acidimicrobiia | Acidimicrobiales | Unknown | Unknown |
| OTU4 | Bacteria | Proteobacteria | Deltaproteobacteria | Syntrophobacterales | Syntrophobacteraceae | Syntrophobacter |
| OTU40 | Bacteria | Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | Candidatus Koribacter |
| OTU42 | Bacteria | Acidobacteria | DA052 | Ellin6513 | Unknown | Unknown |
| OTU45 | Bacteria | Acidobacteria | TM1 | Unknown | Unknown | Unknown |
| OTU49 | Bacteria | Acidobacteria | DA052 | Ellin6513 | Unknown | Unknown |
| OTU5 | Archaea | Crenarchaeota | MBGA | NRP-J | Unknown | Unknown |
| OTU50 | Archaea | Crenarchaeota | MBGA | NRP-J | Unknown | Unknown |
| OTU55 | Bacteria | Acidobacteria | Acidobacteriia | Acidobacteriales | Acidobacteriaceae | Unknown |
| OTU56 | Bacteria | Proteobacteria | Gammaproteobacteria | Xanthomonadales | Sinobacteraceae | Unknown |
| OTU58 | Bacteria | Proteobacteria | Deltaproteobacteria | Syntrophobacterales | Syntrophaceae | Unknown |
| OTU59 | Archaea | Crenarchaeota | MCG | Unknown | Unknown | Unknown |
| OTU6 | Bacteria | Acidobacteria | DA052 | Ellin6513 | Unknown | Unknown |
| OTU69 | Bacteria | AD3 | JG37-AG-4 | Unknown | Unknown | Unknown |
| OTU7 | Bacteria | Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | Unknown |
| OTU74 | Bacteria | Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | Unknown |
| OTU75 | Bacteria | Proteobacteria | Deltaproteobacteria | Unknown | Unknown | Unknown |
| OTU76 | Bacteria | Chlorobi | BSV26 | Unknown | Unknown | Unknown |
| OTU8 | Bacteria | Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | Unknown |
| OTU82 | Bacteria | Proteobacteria | Deltaproteobacteria | Desulfuromonadales | Geobacteraceae | Geobacter |
| OTU86 | Bacteria | Chloroflexi | Dehalococcoidetes | Dehalococcoidales | Dehalococcoidaceae | Unknown |
| OTU9 | Bacteria | Proteobacteria | Alphaproteobacteria | Rhizobiales | Hyphomicrobiaceae | Rhodoplanes |

**Table 7.29-** Taxonomic assignment for microbial species using Blast IDs (Confidence >0.87) for the >1% data set

| OTU | Blast ID | Identity (%) | E-Value |
|---|---|---|---|
| OTU1 | Uncultured Acidobacteria | 98 | 3e-123 |
| OTU2 | Desulfobacca sp. | 96 | 6e-110 |
| OTU3 | Uncultured Thermoplasmata archaeon | 97 | 6e-115 |
| OTU4 | Syntrophobacter sp. | 98 | 3e-118 |
| OTU5 | Uncultured Crenarchaeote | 99 | 5e-126 |
| OTU6 | Uncultured Acidobacteria | 100 | 3-128 |
| OTU7 | Uncultured Acidobacteria | 99 | 1-e126 |
| OTU8 | Uncultured Acidobacteria | 100 | 3-e128 |
| OTU9 | Rhodoplanes sp. | 100 | 3e-128 |
| OTU10 | Methylosinus sp. | 99 | 3e-123 |
| OTU11 | Uncultured Nitrospirae | 100 | 3e-128 |
| OTU12 | Uncultured Betaproteobacteria | 99 | 6e-125 |
| OTU14 | Candidate Division NC10 | 98 | 1e-121 |
| OTU15 | Uncultured Syntrophobacterales | 97 | 6e-115 |
| OTU16 | Bradyrhizobium sp. | 100 | 3e-128 |
| OTU17 | Sulfuricaulis limicola | 99 | 1e-126 |
| OTU18 | Edaphobacter sp. | 98 | 1e-121 |
| OTU19 | Uncultured Verrucomicrobia | 99 | 1e-126 |
| OTU20 | Uncultured Phycisphaerae | 92 | 2e-90 |
| OTU21 | Uncultured Acidobacteria | 99 | 1e-126 |
| OTU22 | Uncultured Acidobacteria | 99 | 3e-123 |
| OTU23 | Uncultured Acidobacteria | 98 | 3e-118 |
| OTU25 | Dehalogenimonas sp. | 93 | 2e-99 |
| OTU27 | Uncultured Actinobacteria | 100 | 3e-128 |
| OTU28 | Conexibacter sp. | 100 | 3e-128 |
| OTU30 | Alkaliflexus sp. | 100 | 3e-128 |
| OTU34 | Candidatus Koribacter sp. | 99 | 6e-125 |
| OTU35 | Syntrophus sp. | 98 | 3-118 |
| OTU36 | Aciditerrimonas sp. | 97 | 4e-117 |
| OTU40 | Uncultured Acidobacteria | 100 | 3e-128 |
| OTU42 | Uncultured Acidobacteria | 100 | 3e-128 |
| OTU45 | Uncultured Acidobacteria | 100 | 3e-128 |
| OTU49 | Uncultured Acidobacteria | 100 | 3e-128 |
| OTU50 | Uncultured Crenarchaeote | 97 | 6e-115 |
| OTU55 | Uncultured Acidobacteriaceae | 100 | 3e-128 |
| OTU56 | Uncultured Gammaproteobacteria | 99 | 6e-125 |
| OTU58 | Syntrophus sp. | 99 | 1e-126 |
| OTU59 | Uncultured Crenarchaeote | 99 | 1e-126 |
| OTU69 | Uncultured Bacterium | 97 | 1e-116 |
| OTU74 | Uncultured Acidobacteria | 99 | 6e-125 |
| OTU75 | Uncultured Syntrophaceae | 98 | 2e-120 |
| OTU76 | Uncultured Chlorobi | 87 | 1e-71 |
| OTU82 | Geobacter sp. | 99 | 1e-126 |
| OTU86 | Uncultured Chloroflexi | 96 | 6e-110 |
| OTU98 | Uncultured Acidobacteria | 99 | 3e-123 |

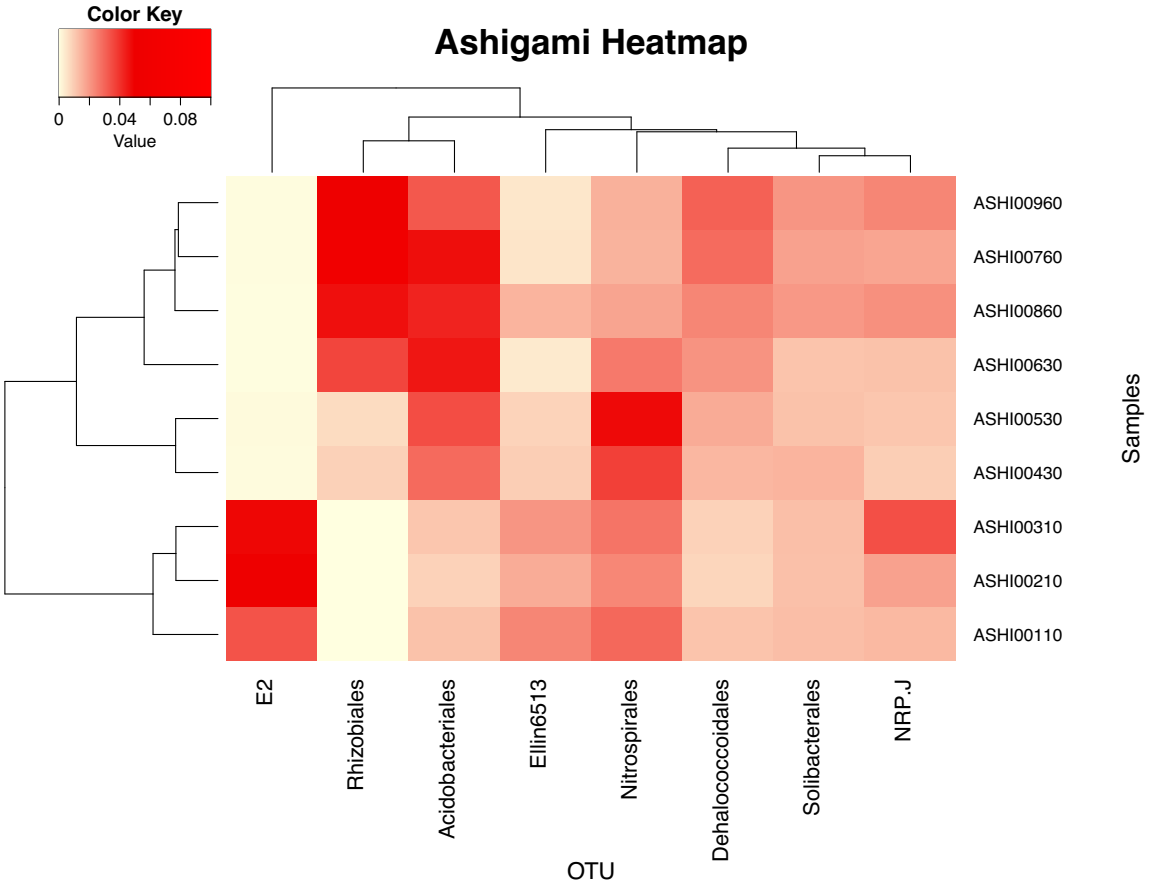



**Figure 7.41-** Heat map at the Ashigami site showing OTUs abundance at the order level across all three depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

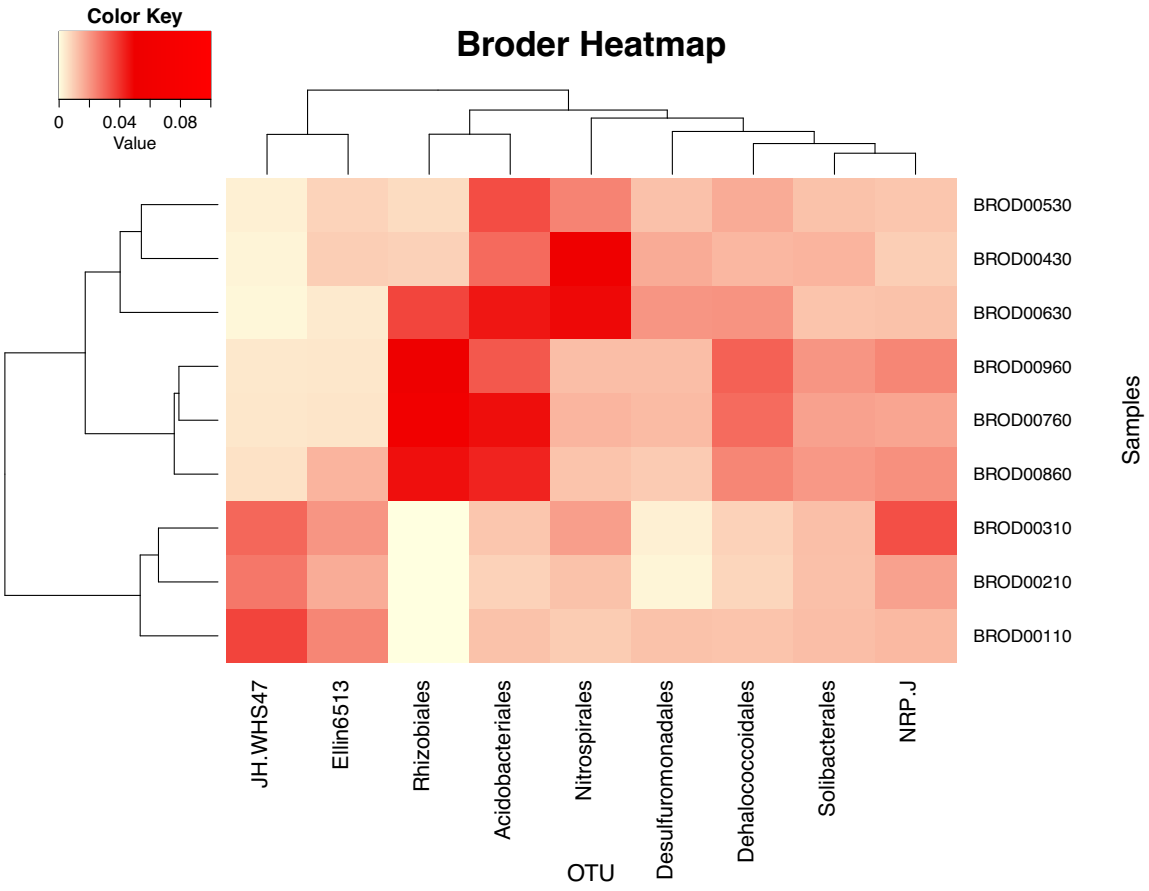



**Figure 7.42-** Heat map at the Broder site showing OTUs abundance at the order level across all three depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

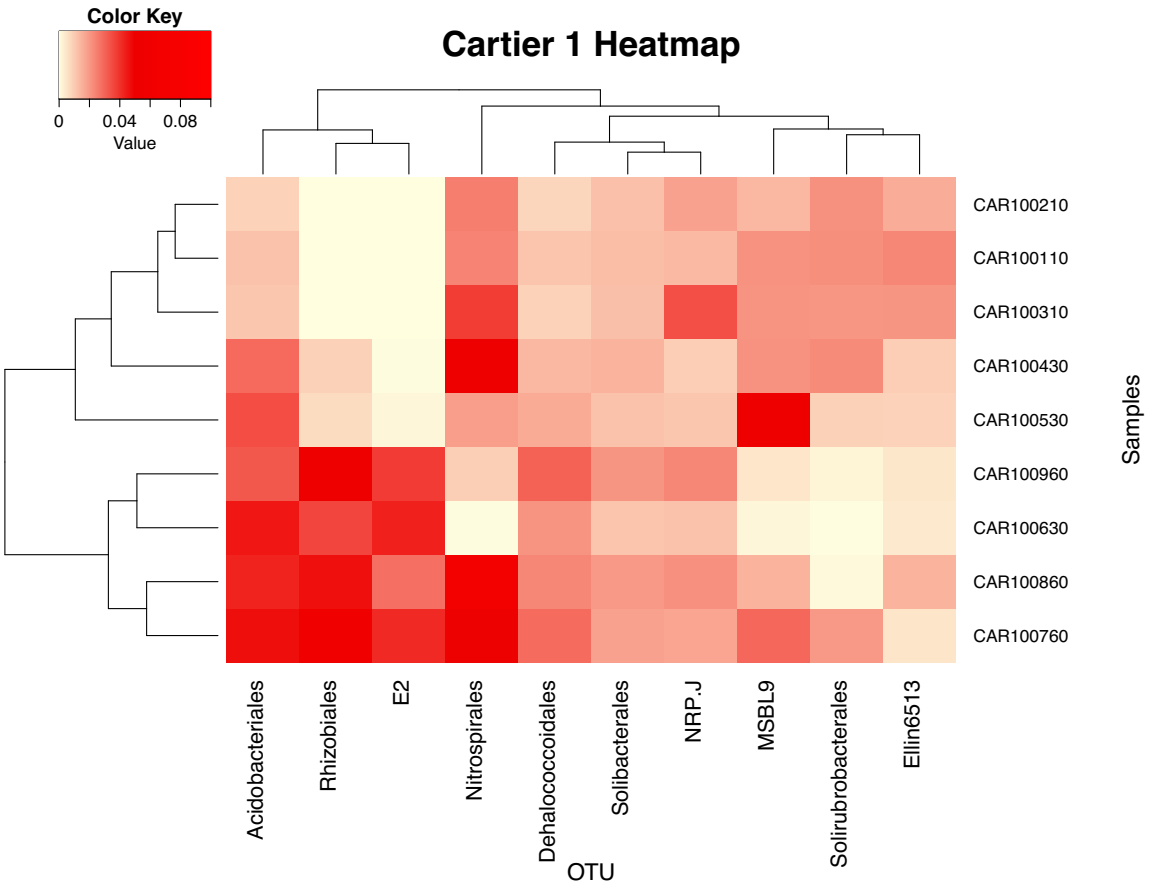



**Figure 7.43-** Heat map at the Cartier 1 site showing OTUs abundance at the order level across all three depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

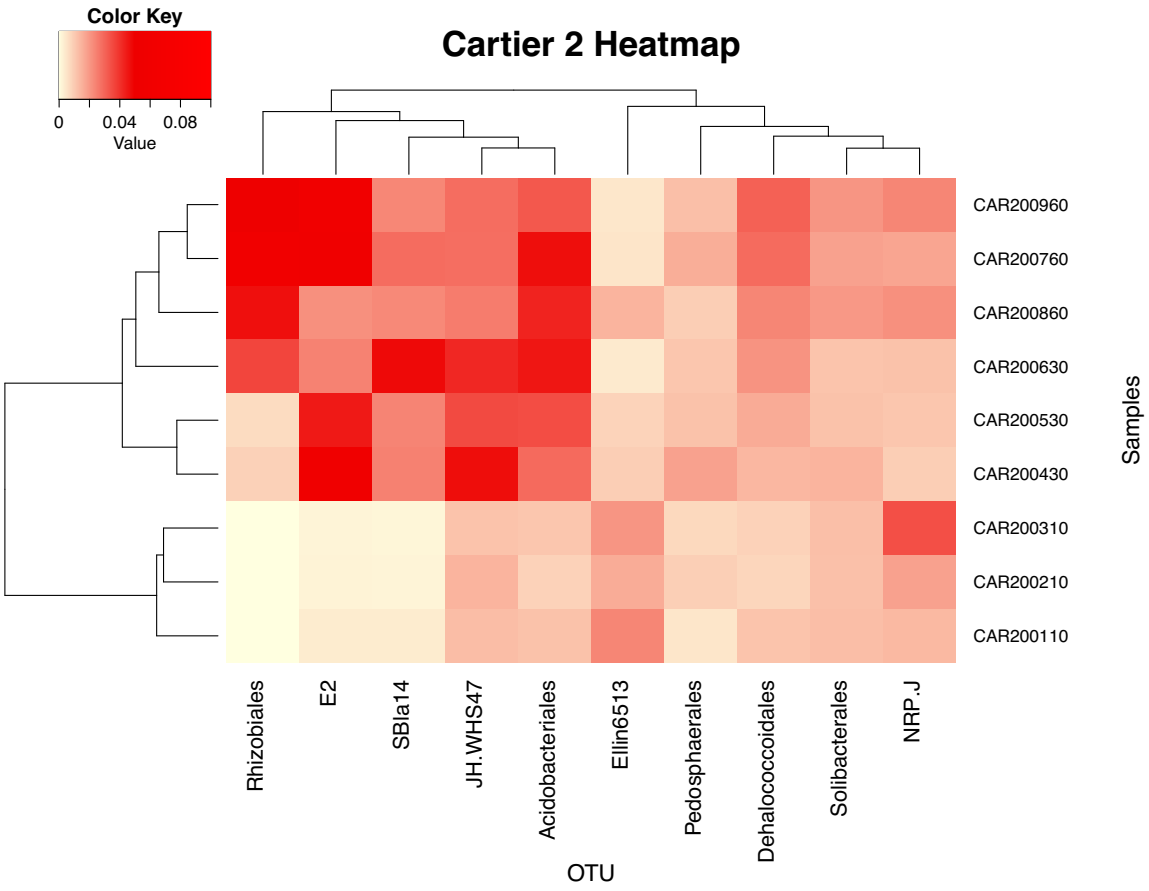



**Figure 7.44-** Heat map at the Cartier 2 site showing OTUs abundance at the order level across all three depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

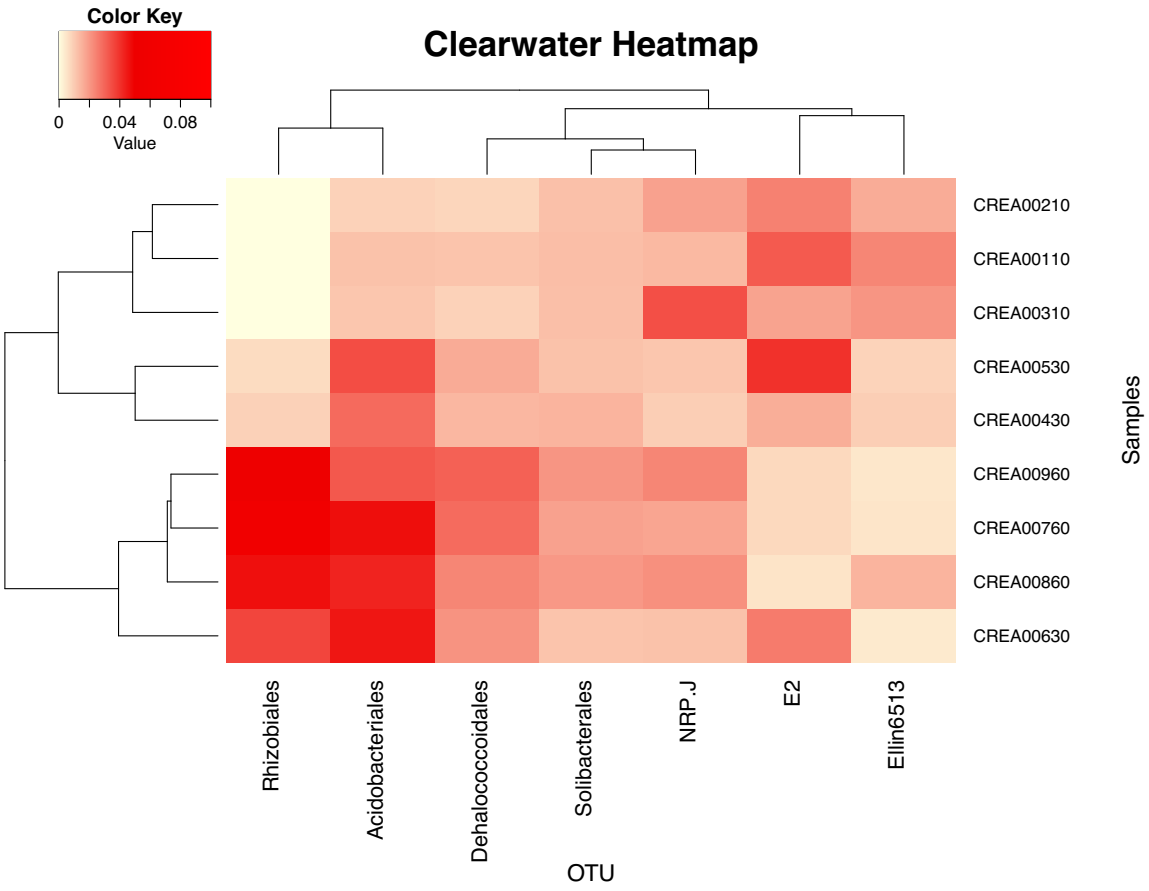



**Figure 7.45-** Heat map at the Clearwater site showing OTUs abundance at the order level across all three depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

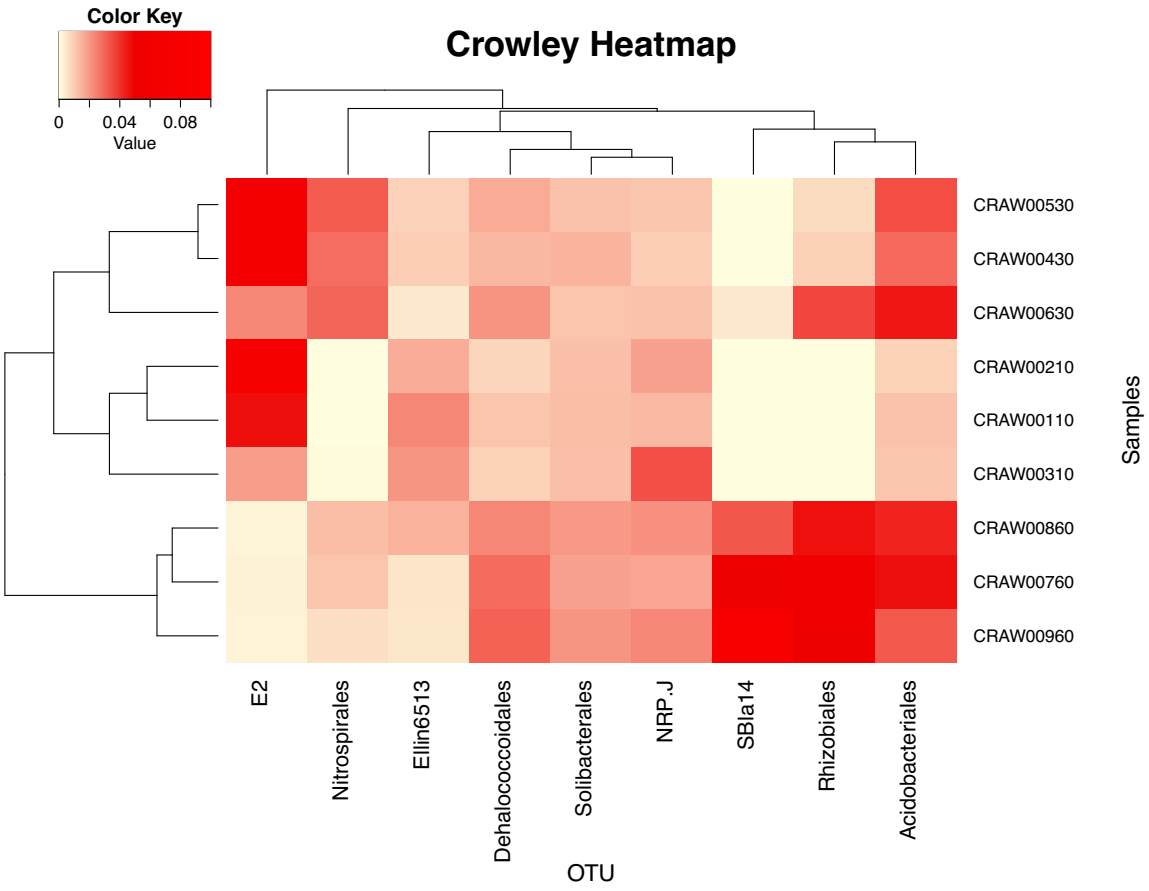



**Figure 7.46-** Heat map at the Crowley site showing OTUs abundance at the order level across all three depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

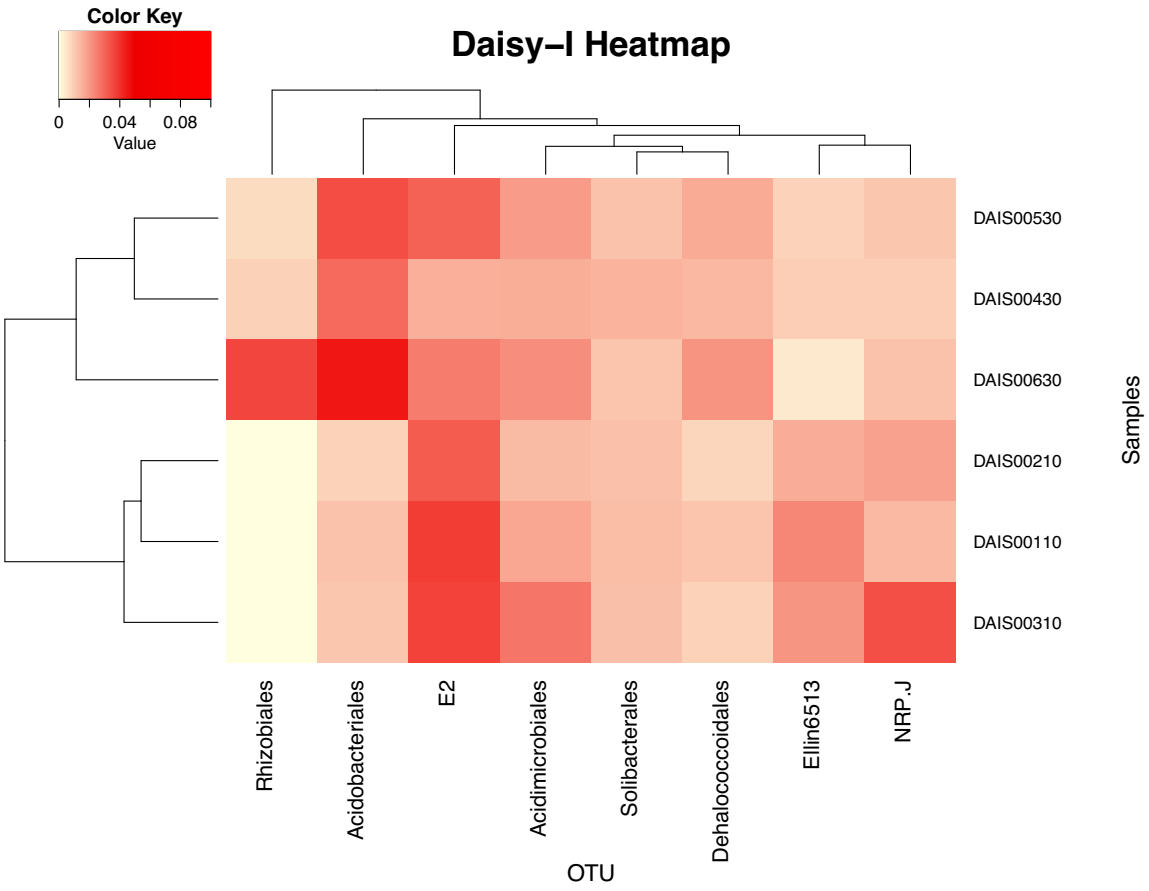



**Figure 7.47-** Heat map at the Daisy I site showing OTUs abundance at the order level across all two depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

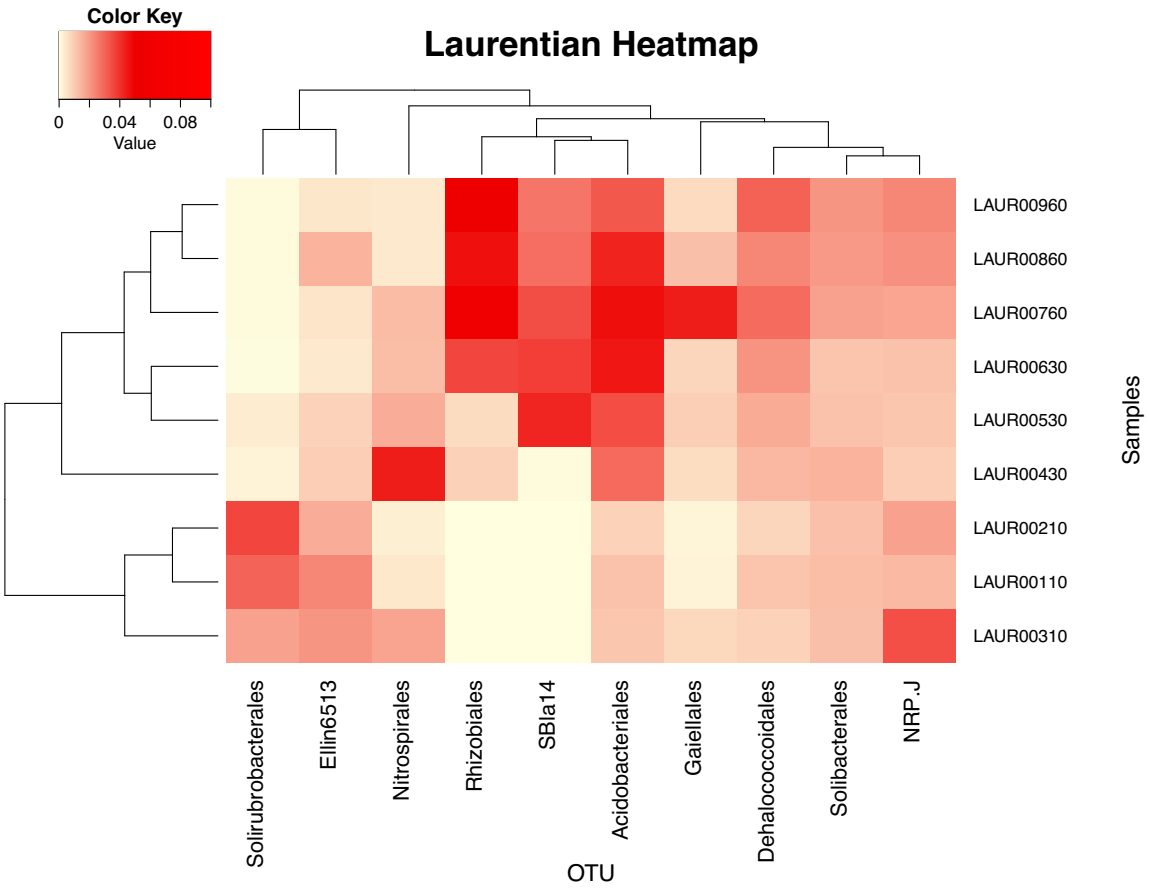



**Figure 7.48-** Heat map at the Laurentian site showing OTUs abundance at the order level across all three depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

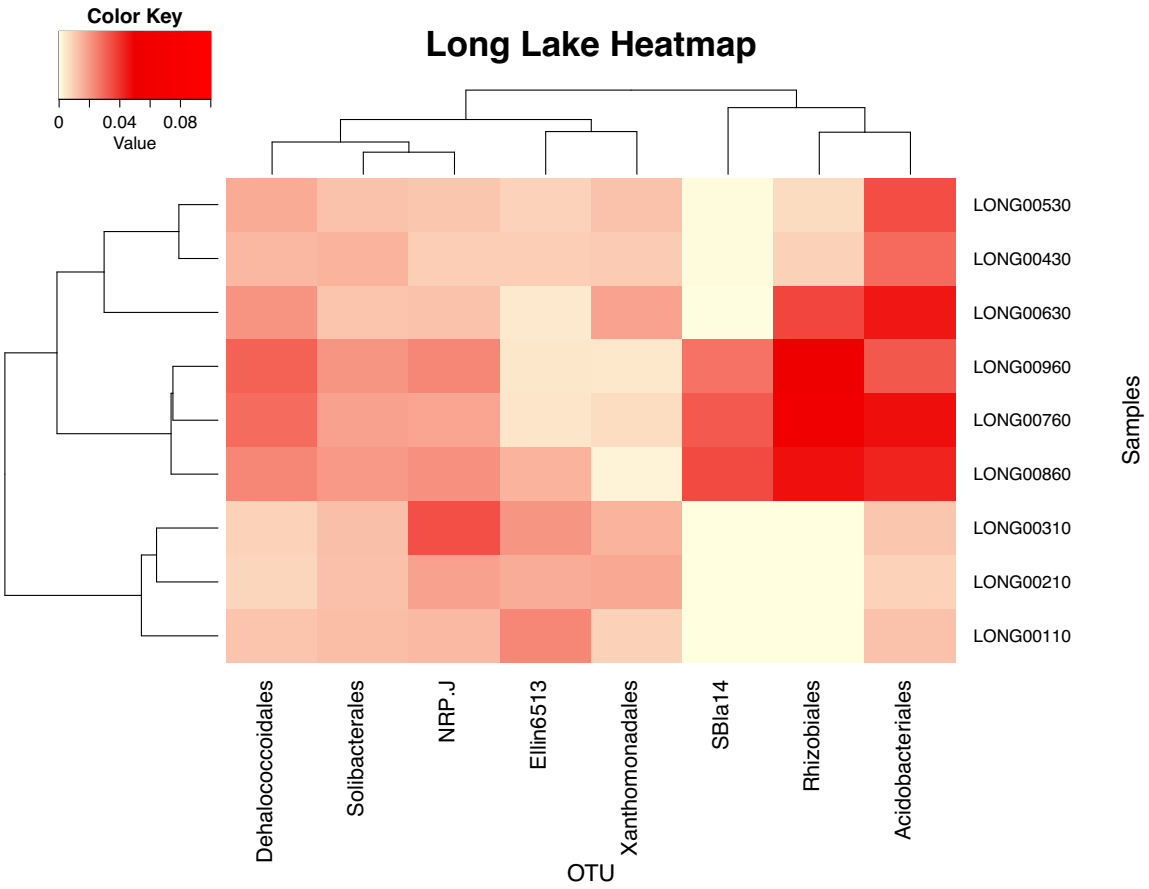



**Figure 7.49-** Heat map at the Long Lake site showing OTUs abundance at the order level across all three depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

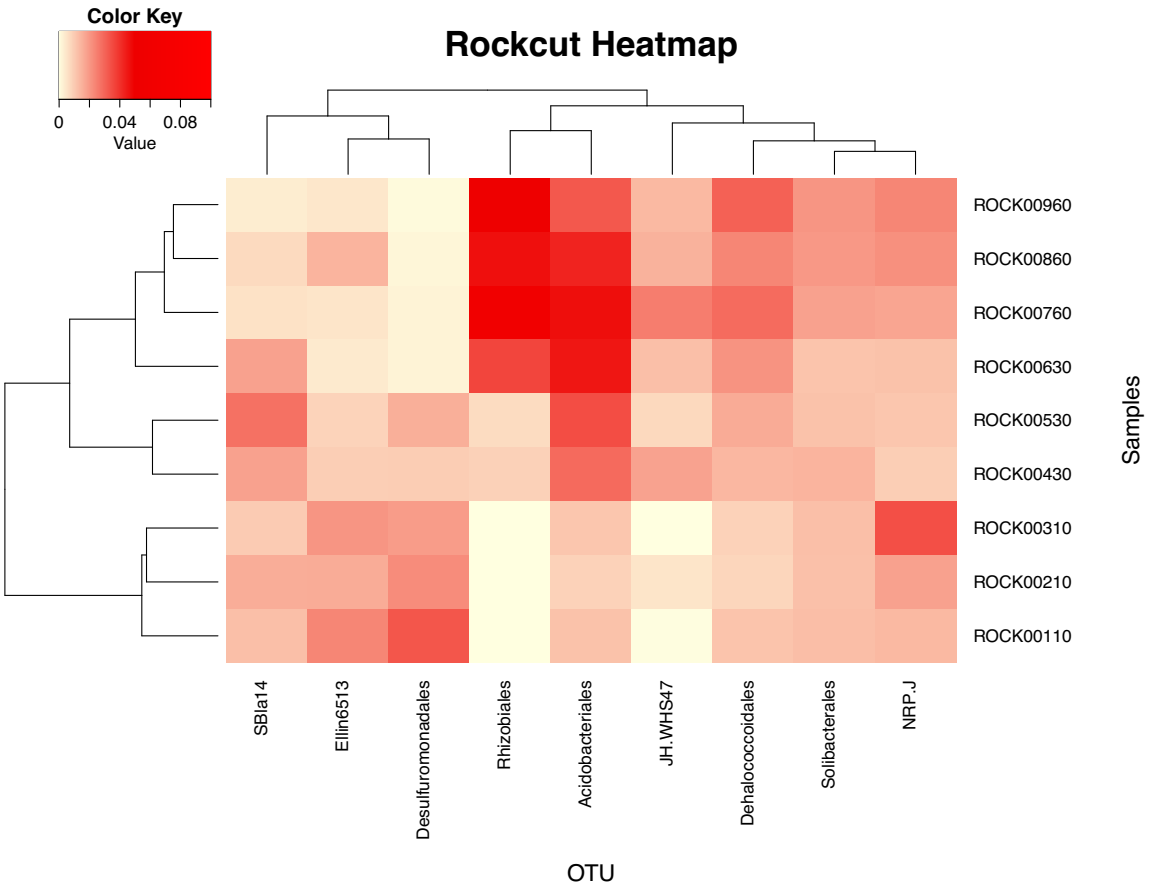



**Figure 7.50-** Heat map at the Rockcut site showing OTUs abundance at the order level across all three depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

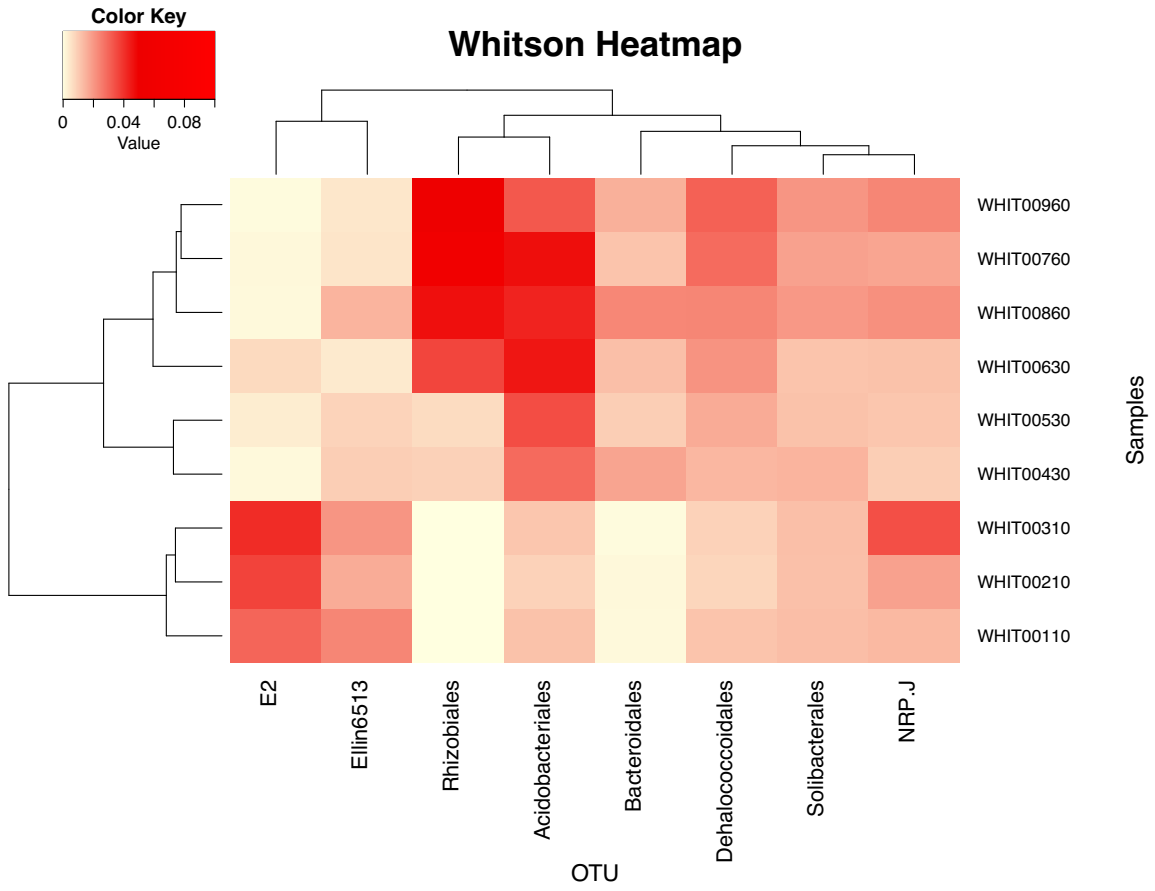



**Figure 7.51-** Heat map at the Whitson site showing OTUs abundance at the order level across all three depths and three plots. The darker color represents higher abundances and the lighter color represents lower abundances. OTUs were clustered using the Bray-Curtis method along both axes.

## 7.8 Microbial Enzymatic Function

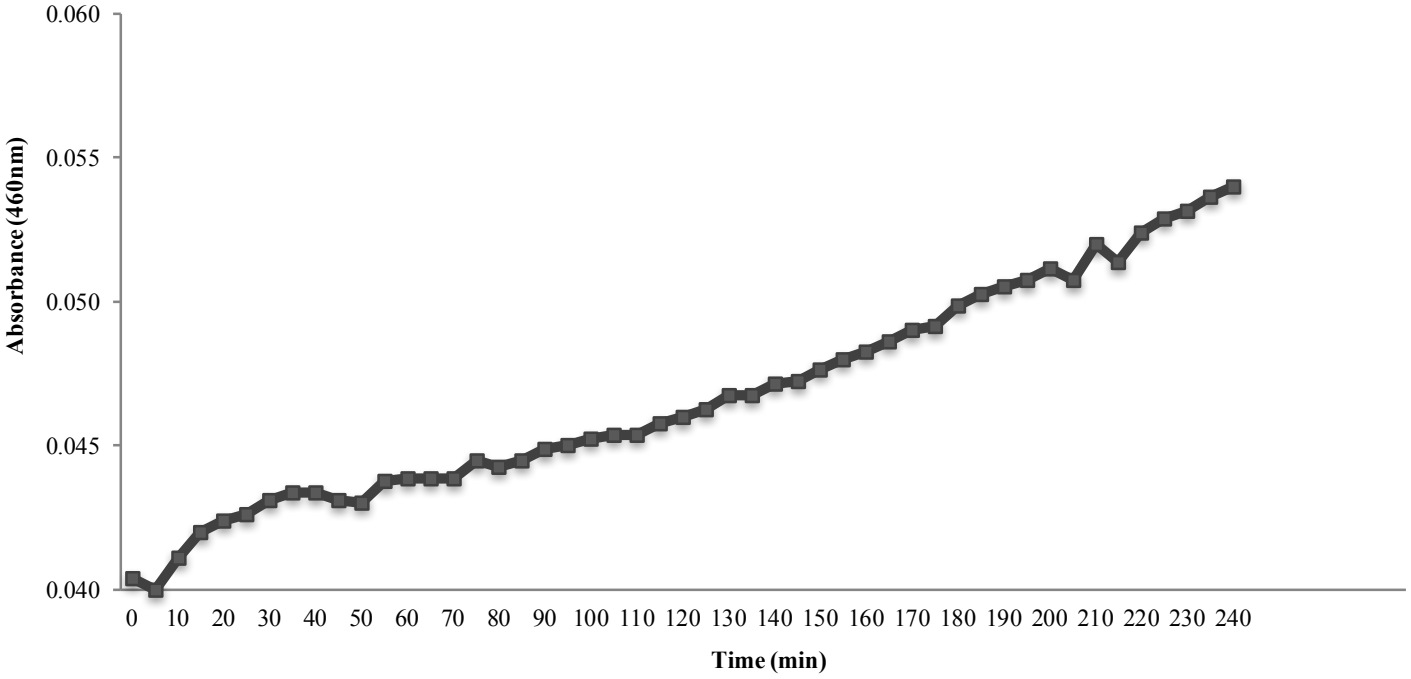



**Figure 7.52**- Peak absorbance curve of LDOPA (2-carboxy-2, 3-dihydroindole-5, 6-quinone) at 460nm over 4 hours. Absorbance curve is used to measure extinction coefficient for oxidase assays (per-oxidase and phenol-oxidase). The extinction coefficient (EC) = OD/c*l, where OD= peak absorbance, c= concentration (M) of LDOPA and l= length of light path. EC = (0.054/(0.05M*0.676cm)), therefore, EC = 1.60 $M^{-1}cm^{-1}$

**Table 7.30**- Six studied enzymes, substrates used and their functions of the enzymes (Sinsabaugh, 2009a; Dunn et al., 2014).

| Enzyme | Substrate | Function |
|---|---|---|
| β-D-glucosidase | MUB-β-D-glucopyranoside | Hydrolysis of glycosidic linkages in carbohydrates |
| Phosphatase | MUB-Phosphate | Phosphomonoester degradation |
| Arylsulphatase | MUB-sulphate potassium | Degradation of arylsulphatase ester groups by the removal of sulphate ion |
| N-acetyl-β-D-glucosaminidase | MUB-N-acetyl-β-D-glucosaminidase | Breaks down chitin polymers |
| Phenol-oxidase | LDOPA | Polyphenol oxidation for lignin |
| Per-oxidase | LDOPA + Peroxide | Polyphenol oxidation for lignin |

**Table 7.31-** Mean per-oxidase and phenol-oxidase activity across the 11 peatlands along the metal gradient

| Sites | Mean Peroxidase Activity (umol/h/g) | +/- SD | Mean Phenoloxidase Activity (umol/h/g) | +/- SD |
|---|---|---|---|---|
| Cartier 1 | 9 | 1 | 11.2 | 0.9 |
| Cartier 2 | 9 | 0.5 | 11 | 0.5 |
| Ashigami | 14.2 | 3.4 | 11.7 | 1.3 |
| Rockcut | 12.1 | 5.6 | 12.4 | 4.7 |
| Broder | 12.4 | 3.6 | 10 | 2.2 |
| Daisy I | 13.6 | 2.7 | 11.8 | 1.6 |
| Crowley | 11.1 | 2.4 | 10 | 2.8 |
| Long Lake | 14.5 | 2.6 | 11 | 2.1 |
| Laurentian | 11.7 | 4.1 | 10.5 | 2.5 |
| Clearwater | 13.1 | 4.8 | 10 | 2.4 |
| Whitson | 15.7 | 5.6 | 10.6 | 3.6 |

**Table 7.32-** Mean hydrolase activity across the 11-peatland sites along the metal gradient

| Sites | Mean Glucosidase Activity (nmol/h/g) | +/- SD | Mean Glucosaminidase Activity (nmol/h/g) | +/- SD | Mean Phosphatase Activity (nmol/h/g) | +/- SD | Mean Arylsulphatase Activity (nmol/h/g) | +/- SD |
|---|---|---|---|---|---|---|---|---|
| Cartier 1 | 1.569166667 | 1.999615683 | 1.443333333 | 1.701487763 | 4.101666667 | 3.72284797 | 16.1425 | 4.43206934 |
| Cartier 2 | 0.8525 | 0.512606973 | 0.676666667 | 0.510371226 | 2.935 | 1.94507537 | 17.355 | 3.424230504 |
| Ashigmai | 2.735833333 | 2.908381175 | 1.316666667 | 1.760161838 | 5.24 | 4.42171501 | 16.88416667 | 3.002451902 |
| Rockcut | 1.803333333 | 1.436215566 | 2.0025 | 2.602429372 | 14.47 | 15.0692746 | 17.79 | 3.591846828 |
| Broder | 2.905 | 3.676682052 | 0.699166667 | 1.024397458 | 5.67 | 6.6732737 | 18.1575 | 1.633591275 |
| Daisy I | 2.535555556 | 1.340458048 | 0.874444444 | 0.834941781 | 6.662222222 | 3.0452577 | 18.51777778 | 2.908091203 |
| Crowley | 2.118333333 | 2.444272836 | 1.233333333 | 2.156526388 | 6.790833333 | 7.98096765 | 17.0625 | 4.714343924 |
| Long Lake | 2.42 | 2.419222264 | 0.8475 | 0.939914164 | 4.2625 | 5.40425611 | 16.6675 | 5.44918697 |
| Laurentian | 0.936666667 | 1.565836014 | 0.936666667 | 2.511349993 | 6.991666667 | 8.32390624 | 14.34416667 | 7.07967829 |
| Clearwater | 2.608333333 | 4.118337779 | 1.2925 | 2.332763188 | 2.521666667 | 4.96140895 | 18.4675 | 1.774358399 |
| Whitson | 5.365 | 6.842861576 | 0.984166667 | 1.050068901 | 4.063333333 | 4.32067405 | 17.43833333 | 1.896886604 |

## 7.9 Environmental Monitoring

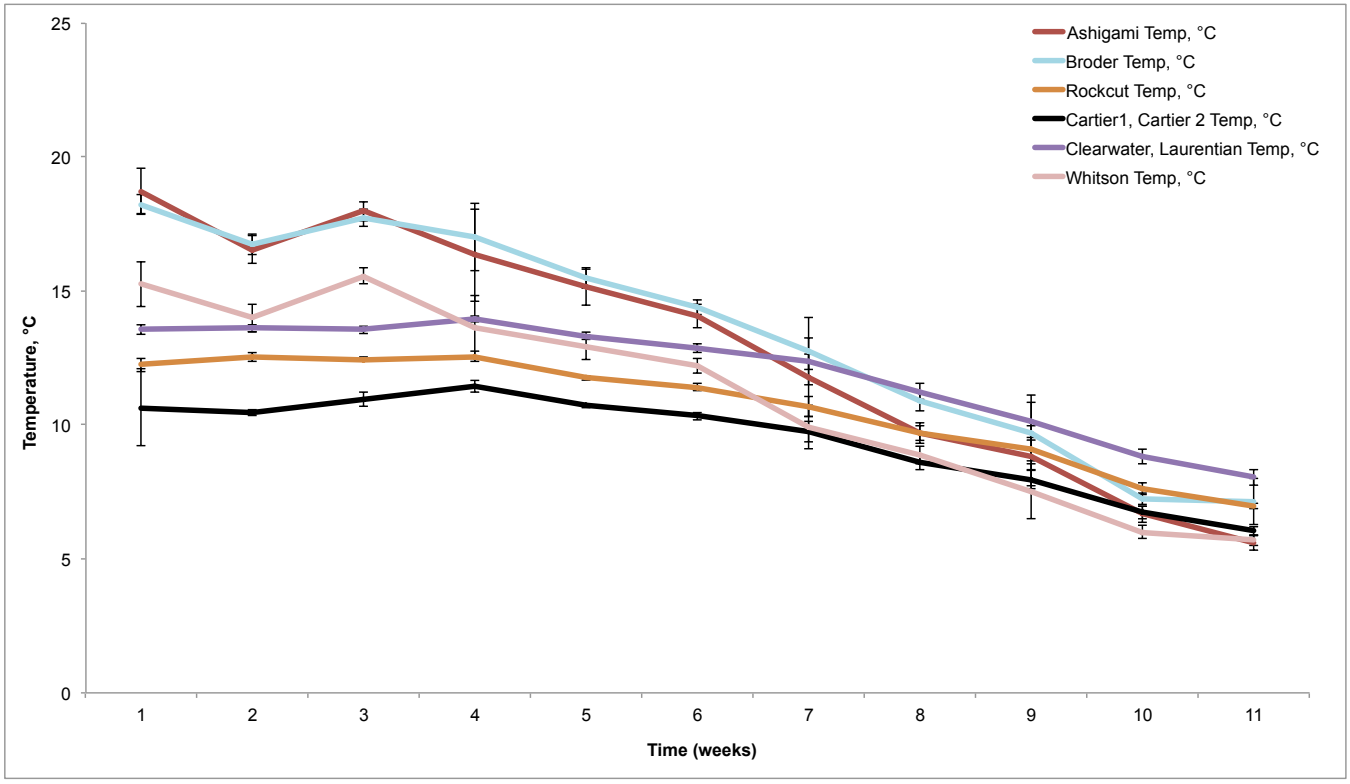



**Figure 7.53**- Changes in mean temperatures (degrees Celsius) across eight peatlands over an 11-week time period from August to November 2015. Temperature loggers were placed approximately 15-cm below the peat surface. Error bars represent standard error of the mean.

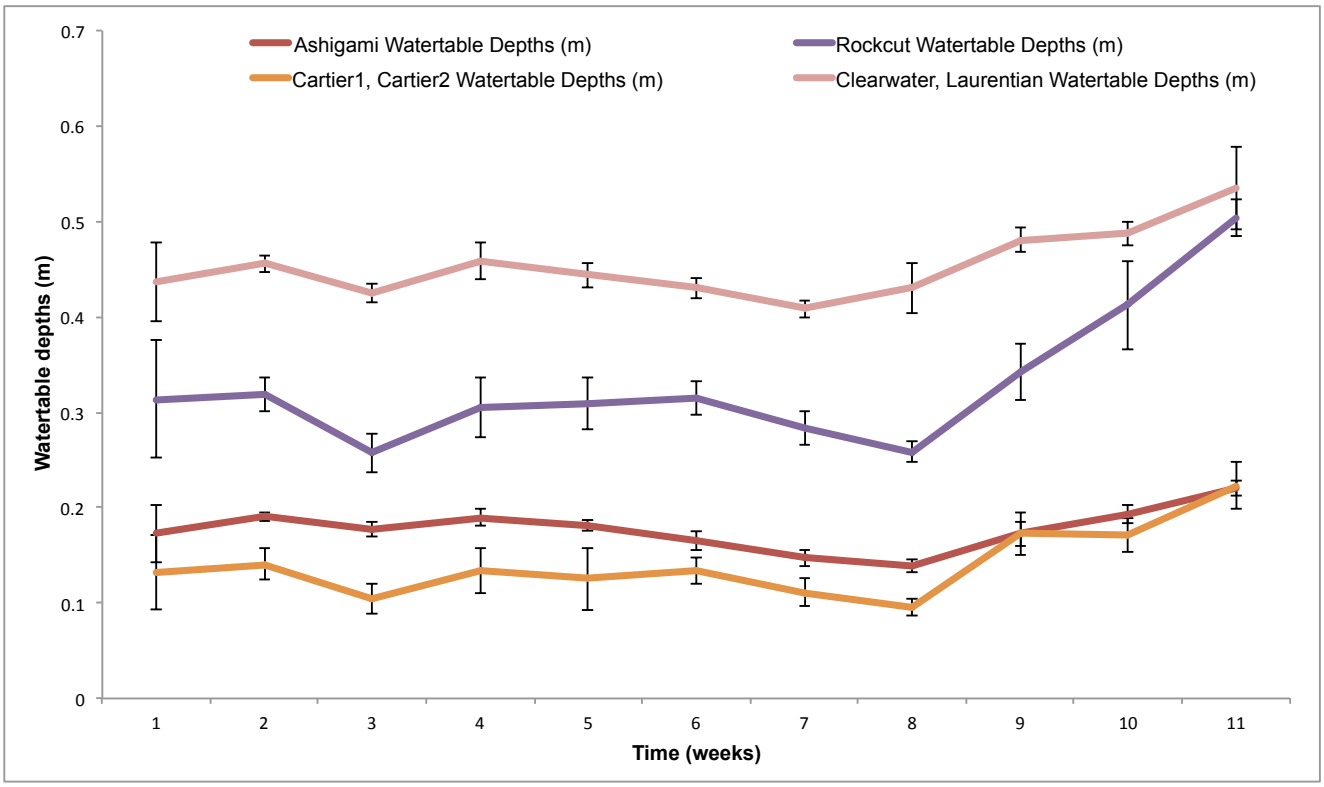



**Figure 7.54-** Changes in mean watertable depths (m) across eight peatlands over an 11-week time period from August to November 2015. Watertable loggers were placed 50-cm below the peat surface. Error bars represent standard error of the mean.